NORTHERN

Utilization Research and Development Division

MARKETING AND NUTRITION RESEARCH

Progress Report

July 1, 1970

AGRICULTURAL RESEARCH SERVICE
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CONTENTS

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PROGRESS REPORT OF THE
NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
MARKETING AND NUTRITION RESEARCH
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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of five research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, the Southeastern at Athens, Georgia, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, grain sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation.

The summaries of progress of research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to those having a special interest in the development of public agricultural research programs.

This report also includes a list of publications and patents issued between April 1, 1969, and June 30, 1970.

Following are some of the recent utilization research accomplishments of the Northern Division.

Starch-Filled Powdered Rubber. Department scientists have developed starch-filled powdered rubbers which, like powdered plastics, can be

molded or extruded without prior high-shear milling to give high-quality finished products. Industry estimates that use of powdered rubber would reduce processing costs at least 2-1/2 cents per pound of finished rubber goods by eliminating much of the heavy-duty equipment and power needed to incorporate curing and reinforcing agents, fillers, and other additives into slab rubber. In the new technology, powdered rubber is merely blended with the other ingredients and final mixing takes place during molding or extrusion. Without the addition of our starch products, rubber cannot be produced in powdered form except by costly means such as grinding in the frozen state.

Currently, mechanical rubber goods, footwear, and miscellaneous types of molded rubber articles constitute a 3.4-billion-pound-per-year market in the U.S. Use of starch-filled powdered rubbers in only half of this market would consume approximately 250 million pounds of starch and save over $40 million per year.

Food Applications Expand Markets for Microbial Gums. Food and Drug Administration approval for use of xanthan gum, a microbial polysaccharide produced from corn sugar, in human foods opens a large new market that is being rapidly exploited by industry. This product of USDA research has been widely acclaimed in superlative terms by commercial developers for its unique combination of properties in aqueous systems. Effective in low concentrations, it forms stable suspensions of solids and oils, it makes thick solutions or gels, which flow when sheared; and it imparts excellent mouthfeel and flavor release to foods and drinks. Outstandingly significant is the lack of change in these properties by heat or cold, freezing and thawing, acids or brines.

Xanthan gum is partially replacing expensive imported plant gums in the food market and extending into new use areas heretofore unfilled. It is expected to be a favored ingredient in food products such as: oil dressings that pour equally well cold or hot; dry sauce and gravy mixes for meats that are stable when heat-sterilized; frozen and thawed, or held on steam tables; an inexpensive, low-calorie instant pudding mix made without egg yolks or fat; and high-viscosity products that must flow easily when pumped.

Xanthan gum was discovered by USDA scientists in a program designed to find new-type polysaccharides, produced from corn sugar by microorganisms. Its commercial development represents an entirely new type of industry in the U.S. Other microbial polysaccharides discovered by USDA scientists and having distinctive properties are under industrial evaluation that might lead to their adoption, also, in the food and other industries.

Progress in Biological Control of the Japanese Beetle. Bacillus popilliae is a bacterium causing milky disease of Japanese beetle grubs. In earlier phases of research to find ways to produce spores of this pathogen for

use as a natural biological insecticide, Department scientists developed techniques for the isolation and maintenance of the pathogen in pure culture; investigated its growth characteristics and mass propagation; evaluated infectivity of artificially propagated cells; studied the chemistry of normal and diseased insect blood; and differentiated metabolism of the host from that of the pathogen. The major remaining unsolved problem was quantity production of spores in artificial medium.

Department scientists have now discovered and isolated several new strains of B. popilliae that produce 30 percent spores. Knowledge gained from study of these strains is expected to result in techniques for production of large quantities of spores suitable for commercial use.

The Japanese beetle is a serious threat to American agriculture. Over 330,000 square miles of the United States are now infested, and an estimated 25 million dollars' damage is caused annually to crops, orchards, pastures, lawns, and ornamental plants. Although B. popilliae spores have the characteristics of an effective biological control agent, the natural spread of milky disease is much slower than that of the insect infestation because the disease affects only grubs, which live underground. Therefore, dissemination of large quantities of spores is needed.

New High-Yielding Source of L-Asparaginase. Survey of microorganisms in the ARS Culture Collection at the Northern Division has led to the discovery of a microorganism, Erwinia aroideae, which produces six times more L-asparaginase than reported from other sources. Besides giving higher yields of L-asparaginase, E. aroideae is also superior to the other sources of the enzyme because it produces only one type of L-asparaginase and not other forms of the enzyme. Recently, L-asparaginase was shown by workers elsewhere to be remarkably effective against one form of cancer, leukemia. L-asparaginase is an enzyme which destroys the amino acid, asparagine, without which the cancerous cells cannot live; normal body cells do not require this amino acid. Up to now, the main source of L-asparaginase was the bacterium, Escherichia coli, and yields were limited. A successful fermentation based on a glucose yeast extract medium was developed to produce the enzyme from E. aroideae. Because the NU enzyme is immunologically distinct, it can be used in patients who become allergic to the E. coli enzyme during chemotherapy. Animal tests indicate the new enzyme source is effective in reducing leukemia in experimental animals. Recently, the National Cancer Institute has requested quantities for tests with primates. Application for a public-held patent has been made.

Linseed Oil for Curing and Protecting Concrete. Department scientists have developed a linseed oil emulsion which when applied to freshly laid concrete retains sufficient water in the concrete for normal curing and, at the same time, protects the concrete from subsequent deterioration in winter on exposure to freeze-thaw cycles and deicing chemicals. This emulsion has been used successfully on a commercial scale for curing and protecting concrete in Kansas, Oklahoma, and Texas. Four companies are now manufacturing this emulsion.

When concrete is laid, it is necessary to cover the surface to retain the moisture needed for proper curing. This protection is needed for about 14 days. One of the covering agents used has been a spray-on coating. Previous work showed that linseed oil coatings were effective in protecting concrete from winter damage, particularly when salt was used. The oil could be applied over the spray-on curing coating. The oil was usually applied 1 month after the concrete was placed and resulted in added expense to the contractor. Department scientists found that linseed oil could be used to give the surface protection needed during curing. Curing and protecting concrete with linseed oil emulsions is likely to result in the use of oil on new highways as well as the specialized structures, and should increase substantially the consumption of linseed oil. Improved emulsions that perform better as curing agents may greatly extend the use of linseed oil for this purpose. Flaxseed from which linseed oil is obtained is an important crop in the States of Minnesota, North Dakota, South Dakota, and Texas.

High-Protein Instant Beverage Powder. The need for dietary protein in worldwide child and infant feeding programs will become increasingly critical due to the inadequate production of milk in developing countries and the decreasing availability of nonfat dry milk solids for world distribution.

A process has been developed by Department engineers that uses an improved extrusion-cooked full-fat soy flour to make a spray-dried beverage base which reconstitutes with water to a nutritious drink. The process destroys fat-oxidizing enzymes and antigrowth factors and debitters soy protein to provide good flavor, stability, and nutritional value. The powder made by a combination of extrusion-cooking, wet-milling, and spray-drying steps contains 33 percent protein, 30 percent fat, and 28 percent carbohydrate. The final formulation is based on a blend of the flour (74%) plus hydrogenated soybean oil, sugar, emulsifier, flavoring, vitamins, and minerals. A significant increase in nutrients over those in conventional soybean milks used traditionally in the Orient has been achieved in the new product by the mineral and vitamin fortification. The dry base has good shelf life and reconstitutes to an opaque creamy liquid with good flavor and beverage characteristics.

A cost study shows that with soybeans at $2.70 per bushel, the beverage can be produced for about 10¢ per gallon to which would be added profits and costs of marketing.

Seed Sources for New Drug. Five potential seed sources for L-dopa have been found by Department scientists in a survey of over 1,000 plant species. This medicinal agent, whose effectiveness was discovered in 1967 at Brookhaven National Laboratory, dramatically alleviates symptoms of most cases of Parkinsonism and offers new hope to 0.5 to 1.5 million Americans who suffer from this disease. Although L-dopa is currently

being produced synthetically, a natural source is preferable because the synthetic procedure gives a mixture of equal amounts of the medically useful L-dopa and an inactive D-form. Separation of the two forms is difficult and expensive. Several companies are therefore interested in developing natural sources for L-dopa and one has patented a recovery process.

The five seed sources are all velvet beans and close relatives. Velvet beans are currently grown on farms primarily for green manure or grazing.

Unexpected Chemical Variety Found in Seed Oils of Uncultivated Plants

In a search for new industrial oilseed crops, Department scientists have discovered in natural seed oils 52 new fatty acids, many of which occur in amounts large enough to be practical sources of industrial raw materials. Still other seed oils containing previously known, useful fatty acids have been found in species potentially adaptable for growth in the United States. Limited commercialization of crambe oil, a rich domestic source of erucic acid used in lubricants and the plastics industry, has already been achieved. Vernonia, whose oil is an effective plasticizer, is undergoing agronomic evaluation and development. Other oils show promise as bread improvers; intermediates for drug synthesis; and possible replacements for sperm oil. New oilseed crops yielding oils and fatty acids noncompetitive with those from presently available domestic oilseeds will enable farmers to achieve more economic use of their land in response to the varying needs of the nation and will help reduce dependence on foreign sources for critical raw materials.

CORN UTILIZATION

Problems and Objectives

Corn is the major source of the 3 billion pounds of cereal starches and flours used annually in the U.S. for industrial purposes. The constant threat to these agricultural outlets by synthetic products from nonagricultural sources requires continuous effort to develop new and improved products that are capable of doing a better job in industrial products such as paper, chemicals, adhesives, protective coatings, plastics, elastomers, and thickening agents. The use of nearly 80 percent of the U.S. production of corn as animal feed provides direct gains to farmers and to industry through even small improvements in grain quality or processing efficiency. Utilization of corn in foods is also economically important, and significant benefits can be realized from the development of cheaper and more healthful products.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Find new technologies for the conversion of starch by chemical or fermentative processes to products for use in the paper, rubber, chemical, and other industries.

2. Provide increased industrial outlets for new classes of high-amylose corn.

3. Develop low-cost foods from new corn varieties having higher nutritional value than ordinary dent corn.

4. Improve yields and quality of corn dry-milling fractions.

5. Develop more acceptable, varied, and nutritious foods from corn.

6. Remove mold toxins or harmful microorganisms from corn and corn products.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling Artificially Dried Corn

Purpose: Increasing amounts of corn are being harvested by field shelling. Because of its high moisture content, this corn requires

artificial drying to prevent spoilage in storage. Such artificial drying lowers the quality of the corn for dry-milling purposes and results in a lower yield of high-quality grits. A suitable dry-milling process is needed to improve yields, quality, and size of grits.

Progress: For corn artificially dried from 25 to 15 percent moisture, yield of -3-1/2+16 mesh grits fell from 63 to 54 percent and fat content rose from 0.5 to 0.9 percent as drying air temperature was increased from ambient (35-60° F.) to 290° F. Cooked paste viscosity (Amylograph) of flour and grits recovered from this corn was influenced by drying air temperature and reached a maximum for about 225° F. drying air temperature.

The concentration of tocols and tocotrienols was determined in the germ, endosperm, and pericarp from normal dent, early-maturing dent, high-oil, and high-lysine corn types. Concentration of alpha-tocopherol was highest in the germ fraction from the high-lysine corn. On the average, the germ fraction contained approximately 78 percent and the endosperm fraction 19 percent of the total tocopherols. The germ fraction contained 95 percent of the total alpha-tocopherol while the endosperm fractions contained all of the measurable tocotrienols. Tocopherols were also investigated in wet- and dry-milled corn fractions. Although good recovery of dry matter and oil was obtained by both milling operations, some loss of tocopherols occurred during processing. Recovery of alpha-tocol and total tocopherols was 68 percent and 77 percent after dry milling and 18 percent and 29 percent after wet milling. Dry-milled germ contained a higher concentration of tocopherols than did wet-milled germ. No tocopherols were detected in starch, steep-water, or process-water.

2. Conversion of Starch to Sugars and Sirups

Purpose: Use of corn sugar and sirups in foods is increasing. Our research is directed towards development of methods for producing sweeteners from starch on a continuous basis and with fewer reversion products thereby helping to reduce processing costs and to increase quality and effectiveness of the products.

Progress: Starch substrates have been efficiently converted to glucose by glucoamylase immobilized on DEAE cellulose. Thirty percent dextrin substrates were converted to about 99 percent of theory as reducing value in stirred reactors. This performance is well within the realm of a possible useful commercial process.

Glucose isomerase from Streptomyces phaeochromogenes NRRL B-3559 has been successfully entrapped in polyacrylamide gels. The entrapped

enzyme has about 50 percent of the specific activity of soluble enzyme. In batch-type reactors, the immobilized enzyme is fully active for about 3 hours after which the activity rapidly falls off. In columns the enzyme remained fully active during three consecutive runs of 5-6 hours' duration. Conditions for producing 3-ketomaltose from maltose by Agrobacterium tumefaciens have been identified. Four percent maltose medium could be converted to 3-ketomaltose in 38-40 percent yields in 18-20 hours. At 12 percent maltose levels the yield dropped to 25 percent.

Cation and anion exchange resins were surveyed in 10 different ionic forms to determine their suitability for fractionating the sugars of starch hydrolyzates. For separation of dextrose and maltose, insignificant differences were found among the cations, except for Pb^{++}; however, the molybdate form of an anion exchanger showed a promising separation with only water as the eluent. Maltitol (4-O-α-D-glucopyranosyl-D-glucitol) was synthesized by borohydride reduction of the reducing end-group of maltose. Purification through the nonaacetate gave amorphous maltitol suitable for sweetness evaluations. Five men rated 20 percent aqueous solutions of maltitol, maltose, and sucrose in order of sweetness: Three judged maltitol sweeter than sucrose, one rated maltitol as sweet as sucrose, and one thought maltitol was only as sweet as maltose. Maltitol and sucrose were rated definitely sweeter than maltose by four of the five tasters.

Under a grant to the University of Minnesota, Minneapolis, the enzyme profile under each of the four morphological areas of a hyphal branch has been determined. Alpha-amylase is associated with the new growth zone and the zone of older growth next to it. Protease is associated with the intermediate zones. Acid phosphatase is found with the older mycelium. Alkaline phosphatase is associated with the area near the inoculation point.

In grant research at Iowa State University, two lines of L-forms of Bacillus subtilis have been prepared. These forms were found to excrete amylase when grown on starch showing that the cell wall is not essential for amylase formation. The Pseudomonas stutzeri amylase which produces high yields of maltotetraose from starchy substrates has been isolated in crystalline form. Two active fractions were found by gel filtration. The two forms apparently have a monomer-dimer relationship.

3. Low-Cost Institutional Foods From Corn

Purpose: Institutions such as homes for the aged or dependent children, hospitals, boarding schools, prisons, and military

establishments require foods providing maximum nutritional benefit at minimal cost. School lunch and other public nutritional programs make similar food demands. Corn is a promising source of inexpensive food products that can be prepared in a variety of attractive, nutritious, and appetizing ways.

Progress: Degermed corn meal can be fortified with up to 15 percent of defatted soy flour without affecting any of its cooking, baking, nutritional, taste, or storage properties. Fortified corn meal has been found to perform well in gruels or in chapatti and corn bread applications. Specifications have been prepared for government purchase of a high-protein enriched macaroni-type product containing 60 percent corn flour, 30 percent soy flour, and 10 percent wheat flour.

Iron fortification of corn grits at a level of 100 mg. per pound of grits can be conveniently accomplished by using ferric orthophosphate. Fortification of farina with ferric orthophosphate was accomplished at an iron level of 200 mg. per pound, but there was some migration of iron to both the top and to the bottom. Ferric ammonium citrate appears at this time to be the most satisfactory iron salt for use in fortifying corn sirup at iron levels of 100 mg. per pint of sirup. This research on iron-fortified corn sirup was performed to enable specification of such a product for infant feeding studies under a contract at Beth Israel Hospital, Boston, Massachusetts, sponsored by HN. A large quantity of sirup fortified according to our specifications has been prepared, specially bottled, and shipped to the hospital by an industrial corn processor.

Fifty-pound lots of instant cereal-soy-milk blends were prepared for UNICEF from rice and millet as well as corn and sorghum. Similar blends, in which the cereal component was replaced with cassava flour and sweet potato flakes, were also prepared. Evaluation by UNICEF indicated that PER values of these blends ranged from 2.3 to 2.7 (casein, 2.5) and NPU values from 55 to 66. Addition of flavors (coconut, vanilla, and others) to instant food blends is easily accomplished and could be used to enhance their palatability and acceptance by specific consumer groups. Storage stability tests show no significant changes in flavor and odor of instant WSM, and wheat flour and CSM fortified with up to 3 percent tricalcium phosphate.

Drying of corn grain at elevated temperature caused sulfhydryl oxidation and reactions of carbohydrate with lysine that reduced solubility and nutritional value of proteins. Storing high-moisture corn in silos retained corn protein digestibility. A pregelatinized corn starch product of high cold paste viscosity and readily dispersible in cold water has been developed.

Microscopic study of subcellular endosperm protein structure of high-amylose corn showed that the corn was deficient in alcohol-soluble protein bodies. This observation suggests that the protein is of better nutritional quality than that of normal corn. Amino acid analysis showed that the lysine content, 2.9 g./100 g. of protein, was intermediate between ordinary and high-lysine corn. A cross containing recessive opaque-2 and amylose extender genes had vitreous kernels but the starch was similar to that of high-amylose corn. Because of its high gelatinization point, high-amylose starch is nutritionally inferior to dent corn starch.

Thermal decomposition of 1-deoxy-1-prolino-D-fructose at 138° C./1 mm. produced among the volatiles separated by GLC: 1-pyrroline, with corny, amine-like aroma; a fraction with bready and nutty aromas; and a fraction with caramel aroma. Separation and characterization of the caramel aroma compounds showed the presence of a dihydroxy γ-pyrone related to maltol, acetylformoin, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. The critical browning intermediate, 3-deoxy-D glucosone, was isolated along with 1-deoxy-1-n-butylamino-D-fructose from the reaction of D-glucose with n-butylamine acetate.

In corn, three lipid-active enzymes (lipase, lipoxygenase, and linoleate hydroperoxide isomerase) were found to be concentrated in the germ tissue. Conditions of heat used in processing the corn germ were evaluated as to the degree of enzyme inactivation achieved. Heat-treated full-fat flakes of germ were more stable to hydrolytic rancidity when lipase was inactivated. However, heat-treated germ was less stable to oxidative rancidity than unprocessed germ. The oil in unprocessed germ was presumably protected from oxidation through the action of linoleate hydroperoxide isomerase.

A new colorimetric method using dichloroacetic acid has been developed to determine tryptophan in proteins. The procedure is based on the reaction of glyoxylic acid with the indole ring. The reaction does not work on samples containing large amounts of carbohydrates. A number of lysine-infused popcorn samples have been analyzed. It was found that infused lysine is carried into the popped product.

In contrast research at the North Star Research and Development Institute, a new method for estimating lysine in wheat flour, based on formation of a dinitrophenyl (DNP) derivative, was applied to corn. In this analysis, proteins are extracted with dilute aqueous sodium hydroxide. Corn endosperm required stronger alkali than did wheat flour to achieve nearly quantitative (96%) solubilization of proteins, and the resulting extracts were not well suited for the DNP method. A stepwise extraction procedure involving use of 70 percent alcohol followed by dilute aqueous alkali achieved 100 percent solubilization of corn endosperm proteins except for horny endosperm varieties. The combined extracts were suitable for use in the analysis.

Lipoxygenase was shown to be located in germ tissue of corn, and to catalyze the oxidation of linoleic acid to 9-D-hydroperoxy-trans-10, cis-octadecadienoic acid. Lipoxygenase activity was inhibited in full-fat germ by a factor which has not yet been identified.

In grant research at Purdue University, the major emphasis was directed to properties of proteins in high-lysine corns. Protein ranged from 9 to 21.6 percent in a series of opaque-2 crosses while lysine ranged from 4 to 5.2 percent. Samples of opaque-2 hybrids produced in Colombia were high in lysine although their kernels were translucent.

At the University of Missouri, it was found that the phytate bound to the albumin-like protein extracted from corn germ can be dissociated from the protein by trichloroacetic acid and by chromatography on an anion exchange resin. The phytate of corn germ obtained by iron-precipitation appears to be composed entirely of inositol hexaphosphate.

Research on corn foods has continued to produce information of importance to both international and domestic nutritional programs. International importance is attested by the continued interest of UNICEF and, in particular, by its purchase of 115 tons of instant corn-soy mix for relief in the Nigerian emergency. On the domestic front the soy-corn-wheat macaroni-type product is being test marketed by an industrial company. Consideration is being given to government purchases of this product and of soy-flour- and iron-fortified corn grit products.

4. Rennin from Microbial Sources

Purpose: Rennin, which is found in an extract of the stomach lining of milk-fed calves and which is almost universally used in manufacture of cheese, is rising rapidly in price as the availability of the animal source diminishes. An alternate source of a milk-curdling agent is needed.

Progress: Samples of cheeses made at the Eastern Division with the Northern Division's Rhizopus oligosporus protease and with animal rennin were sent to us. The cheese made with our protease was intensely bitter; however, the body and texture of both cheeses seem comparable. Preliminary characterization studies on five active fractions (A - E) of R. oligosporus protease showed that the fractions respond similarly to inhibitors and changes in pH. However, consistent differences in pH optima, temperature optima, and relative activity of casein digestion to milk-clotting are found between two groups (Fractions A, B, and C) and (Fractions D and E). Crystalline enzymes were obtained from Fractions A and B. A laboratory test indicated that Fractions A and B produced intense bitterness in cheese made with them. Studies are in progress to determine if Fractions C, D, and E perform effectively in the cheesemaking process and if their use imparts bitterness to the product.

Nearly all of the 10 strains of Chlamydomucor oryzae in the ARS Culture Collection produced milk-clotting and antibacterial substances. In a survey of 199 strains of Rhizopus, 156 strains produced a milk-clotting substance and 45 strains produced a substance that exhibited antibacterial activity. If a suitable enzyme for cheesemaking is not produced by R. oligosporus, it is possible that one of the other Rhizopus, or Chlamydomucor strains may prove to be a source of a satisfactory enzyme.

The antibacterial compound produced by R. oligosporus has been purified and identified as a glycoprotein. Data from amino acid analysis revealed that the compound contains all the amino acids routinely detected by automatic amino acid analyzer; and data from paper chromatography indicated the presence of galactose. We also have found that antibacterial compounds are produced by other molds commonly used in Oriental food fermentation, e.g., R. chinensis, R. oryzae, R. chlamydosporus, Chlamydomucor oryzae, and Actinomucor elegans.

Grant research at the University of Wisconsin, Madison, revealed that Aspergillus oryzae var. effusus exhibits no spontaneous mutation but auxotrophic mutants can be induced by subjecting conidia to ultraviolet radiation at a wavelength of 2750A.

5. Dry-Milling of High-Lysine Corn

Purpose: High-lysine corn may become the predominant corn within a few years because its protein is more nutritious than that of ordinary dent corn. However, strains of high-lysine corn available to date, such as opaque-2 corn, have essentially none of the horny endosperm needed for producing a high yield of grits by customary dry-milling procedures. Also, prime products from high-lysine corn tend to be of lower quality than those from dent corn; e.g., in a test performed by a commercial milling company, oil, fiber, and ash content of grits was higher than desired. It is, therefore, important to know if conventional milling techniques can be better adapted to high-lysine corn.

Progress: A more satisfactory procedure based on use of conventional equipment was developed for dry-milling opaque-2 corn. Prime products (grits, meal, and flour) of acceptable fat content (max. of 1.0% for mixture) were produced when corn was properly tempered to 24 percent or higher moisture levels. Table grit yield was low (11-14%) and flour yield high because of the floury nature of opaque-2 kernels. No flaking grits were recovered. When degermer fines were included in the prime product mix, total yield of the latter varied between 62 and 72 percent (based on product recovery) and was comparable to or slightly better than that from a normal dent hybrid corn. Protein contents of endosperm fractions generally were lower than those from normal corn.

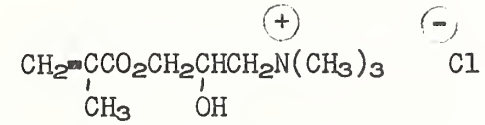
This procedure overcomes some of the difficulties encountered in milling high-lysine corn with conventional techniques but does not eliminate low yields of grits and high yields of flour. It does not appear possible to solve this problem by use of conventional methods. Alternative approaches, such as pretreatments to increase endosperm cohesion or development of high-lysine corn strains having vitreous endosperm, may be required.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Starch Graft Copolymers

Purpose: A large, expanding market exists for water-soluble high polymers as thickening or flocculating agents and as adhesives. Graft copolymerization of starch with commercially available organic monomers affords good opportunities for developing starch-based products that can compete effectively with nonagricultural synthetics in finding outlets in this market.

Progress: Graft copolymers of starch and the cationic monomer



are effective flocculants for finely divided silica. Gel permeation chromatography has shown that the molecular dispersity $\bar{M}_w/\bar{M}_n$ values for the polyacrylonitrile graft chain in starch-polyacrylonitrile (S-PAN) graft copolymers vary markedly according to conditions used in preparing S-PAN. The use of crude, instead of purified, S-PAN in the hydrolytic conversion of S-PAN to a high-quality, water-soluble polymer now appears feasible.

Grafted polymers of starch have been extensively evaluated on the pilot paper machine and found to be as effective as the best commercially available retention aids. Additional benefits were realized in increases in freeness and dry-strength properties.

Under contract research at Stanford Research Institute, Menlo Park, three series of terpolymers were prepared by grafting various amounts of acrylonitrile-butadiene (AN-BD) mixtures to whole starch, 60 fluidity starch, and Amylon VII. Grafted comonomer content of the terpolymers ranged from 10 to 40 weight percent, and the ratio of grafted AN to BD on a weight basis ranged from one-third to one-twentieth. Homopolymer content in the terpolymers was usually on the order of 5 to 6 percent. Selected products were evaluated as wet-web strengthening agents for paper. Although some strength

improvements were obtained, none of the products appears to be effective enough to be of commercial interest. The above starches were grafted with various amounts of AN-styrene mixtures to provide terpolymers ranging in AN-styrene content from 9 to 26 weight percent. As wet-web strengtheners, the products were comparable to the starch-AN-BD-terpolymers.

Synthesis and evaluation of over 50 graft polymers of starch and acrylamide, acrylic acid, acrylonitrile, and quaternary nitrogen olefins have been accomplished through contract research conducted by General Mills, Inc., Minneapolis. Through a combination of extensive laboratory evaluations and limited field testing, five starch graft polymers have been identified which have good potential for industrial acceptance in one or more of the following applications: (a) wet-end additives in making clay-filled papers, (b) flocculation and clarification aids in treatment of industrial and municipal waste waters for process improvement and control of pollution, (c) flotation beneficiation agents in ore refining, and (d) stabilizing agents and thickeners of organic-water mixtures.

At Southern Illinois University, Carbondale, under grant, several benzyl ethers of partially acetylated and unacetylated glucose, as well as benzyl alpha- and beta-D-glucopyranosides and acetates thereof were synthesized and subjected to bromination under free radical conditions. In no case were free radicals detected in the reaction systems by electron paramagnetic resonance spectroscopy. However, with some of the benzyl glucosides, products were isolated which indicate formation of a free radical on the anomeric carbon atom.

2. Starch Xanthides as Wet-Strength Agents in Paper

Purpose: Starch xanthides are promising new products, discovered at the Northern Division, for use as additives to improve strength properties of paper. Successful commercialization of starch xanthides in the expanding paper industry would be an important contribution to overcoming competitive advantages of synthetics and to maintaining and increasing the large volume of starch products now consumed by this industry.

Progress: Practical operating conditions have been established for incorporation of a xanthate-"Kymene" (a commercial polyamide epichlorohydrin condensation product) derivative which improves crush resistance in linerboard when applied at an alkaline pH. Research has progressed to pilot-scale evaluations of this derivative, which appears to be the only known material that will significantly improve crush resistance in linerboard made from pulp under alkaline conditions. Also the derivative needs no further crosslinking. The

improvement in the linerboard is sufficiently great that it would be economically practical to utilize spray-dried xanthate. For these reasons three industrial companies are seriously considering a joint effort to plant-test the new derivative and, if successful, to commercialize it.

Studies on oxidatively crosslinked xanthate and xanthate-polyethyleneimine derivatives are essentially complete. Wet- and dry-tensile strength development is competitive with commercial additives, but process requirements or economics may limit their industrial acceptance. Performance data of commercial wet-end additives are being obtained to provide cost/performance guides for cereal-derived additives.

Reactive epoxide groups have been substituted onto carbohydrates as epoxypropyl side chains. Several multifunctional epoxide derivatives of glucose have been prepared. The epoxide function readily undergoes ring opening with various nucleophiles to provide novel carbohydrate derivatives. Low degree of substitution (D.S.) epoxypropyl derivatives of starch have been prepared. A unique six-membered cyclic carbonate derivative of a sugar has been synthesized. Novel chlorothioformate derivatives of sugars have been obtained from xanthate intermediates.

It has been found, under grant research at Ohio State University, Columbus, that photolysis of primary azide derivatives of starch and cellulose is a useful route for generating an aldehyde function from hydroxymethyl groups in polysaccharides. Characterization of the polyaldehydes as to D.S. is accomplished by a new procedure which employs reduction with borodeuteride, hydrolysis, and mass spectral analysis. A computer program has been developed that facilitates the determination of D.S. of disubstituted polysaccharide derivatives. Amylose has been converted into a 2,3-unsaturated derivative. Bromination of the latter affords an amylose product which has bromine at positions C-2 and C-3 of every glucose unit that was previously unsaturated. Exposure of the unsaturated polymer to acid causes cleavage of the polymer.

In research, now completed, under a PL 480 grant to the Institute of Physical Chemistry, University of Graz, Graz, Austria, standard low-shear, rotational and specially designed high-shear, capillary viscometers were used to characterize the rheological properties of starch pastes up to a shear rate of 10^6 $sec.^{-1}$, which approaches rates existing in commercial coaters. Both time and shear rate dependence of the pastes were determined and the mechanisms responsible for their rheological behavior identified. Methods were then developed for presenting and interpreting the laboratory data that provided unequivocal correlation with end-use performance.

3. Cationic Starches and Flours for Paper

Purpose: Present usage of cationic starches in the paper industry constitutes a sizable starch outlet. To maintain this market in competition with nonagricultural synthetics, either the performance of cationic starches must be improved or the price reduced. A dry process for manufacture of cationic starches would be especially beneficial since production costs are increasing because of pollution problems encountered with the currently used wet processes.

Progress: Two 350-pound lots of aminoethyl corn flour were prepared in the pilot plant by a batch dry-process for use as a wet-end additive in commercial papermaking tests. Both lots performed satisfactorily as retention aids and internal size. A Memorandum of Understanding with Dow Chemical Company permits receipt of information on their continuous process for aminoethylation and provides for production on a semicommercial scale of aminoethyl products for laboratory and commercial tests. Preliminary results indicate that a satisfactory product can be made by this method. Evaluations of cationic aminoethyl flours are continuing on both pilot and commercial scales. Results from mill trials indicate that the cationic flours are competitive with commercial cationic starches in both performance and economics. One large corn-milling company is currently working toward commercial production of aminoethyl corn flour, and a paper manufacturer has expressed interest in eventual use of the product.

4. Starch-Derived Chemicals for Plastics and Coatings

Purpose: The plastics and coatings industries are expanding rapidly. Extensive opportunity exists for products derived from cereal starches and flours to share in this market, thus providing additional benefits to agriculture through new outlets for corn.

Progress: Techniques were established for preparing and evaluating starch derivatives for use in urethane plastic formulations. Preparations were designed to yield products that would have greater reactivity with isocyanates than would pure starch and hence improve the effects of adding starch to plastic formulations. Furthermore, the derivatives were made in aqueous systems to achieve economically feasible products. One derivative, obtained by reacting about 1 mole of benzyl chloride per mole glucose units, showed the greatest promise. It readily reacts with toluene diisocyanate in a nonpolar system. Urethane plastic formulations containing 50 percent of this starch ether yielded plastics with improved transparency and texture over those based on pure starch. Further tests are in progress to determine whether the overall properties of the plastics are improved.

A study was initiated to determine the effect of single and double halogen substitution on the hydrolytic stability of glycosidic bonds. Some 6-mono- and 6,6'-disubstituted methyl β-maltosides were prepared by displacement reaction of the appropriate p-tolylsulfonyl derivatives. Hexamethylphosphoramide was found to solubilize lithium halides in toluene. Reaction of carbohydrate sulfonates with these toluene-solubilized halide ions rapidly produced the corresponding deoxyhalocarbohydrates.

In research under a grant to the University of Pittsburgh, Pittsburgh, Pennsylvania, in order to establish optimum conditions for maximum levoglucosan production from corn starch, pyrolyses were conducted in batches, with and without dielectric heating, in inert gas streams under atmospheric and reduced pressures, with variations in flow rates, charges to the reactor, temperatures of reactor and sweeping gas, type and concentration of catalyst. Different methods for isolating levoglucosan from product sirups were evaluated. Optimum operating conditions involved pretreating corn starch with 10 percent acetic acid solution and drying it before charging 25-50 g. to an oven-heated (400° C.), stainless steel, 2-liter kettle. The reactor was then swept with superheated steam at 350° C., 15-20 ml. condensate/min. at 0.25 atm. pressure to obtain the maximum (44.5%) yield of levoglucosan. Commercial corn flour and corn grits gave lower yields (24 and 15%, respectively); however, porous, friable residues were formed with little or no foaming--an advantage for a continuous process. Optimum separation of levoglucosan from the product sirups involved a 2-stage condensation of vapors and crystallization from prescribed volumes of acetone. Dielectric heating was judged not suitable for batch operation, although it should be applicable in a continuous process. The problems involved in dielectric heating were defined.

At Southern Illinois University, Carbondale, grant research was directed to study of the kinetics of the splitting of various esters of amylose, maltose, and D-glucose in acidic methanol. The methanolyses proceeded much faster than hydrolyses under similar conditions. Two-step degradations of acetic esters were observed with methanesulfonic acid as the catalyst but not with hydrogen chloride. Amylose tribenzoate and maltose octabenzoate were stable to the methanolysis conditions because they resisted deacylation. O-Benzoyl groups at C-2 and C-6 are responsible for protecting the interglycosidic linkages.

Research under a PL 480 grant to the Ahmedabad Textiles Research Association, Ahmedabad, India, showed that protecting the 2,3-hydroxyl groups of starch by carbanilyl groups and hydroxethylating with ethylene oxide under catalysis of sodium bicarbonate-sodium carbonate is a good method for preparing specifically substituted hydroxyethyl ethers of starch. This work has now been completed.

At Jadavpur University, Calcutta, India, under a PL 480 grant, beryllium, magnesium, calcium, strontium, and barium--as hydroxides, alkoxides, or salts--were reacted in aqueous or alcoholic solutions with D-glucose, methyl glucoside, D-glucosamine, maltose, and amylose fraction of corn starch. D-glucose and maltose formed 1:1 addition compounds. Amylose and barium hydroxide formed a complex that contained one mole of barium hydroxide per anhydromaltose unit. New complex salts of maltose with calcium acetate and magnesium methoxide were obtained. Complexing could not be demonstrated between methyl glucoside or D-glucosamine and the alkaline earth metal salts; however, two moles of D-glucosamine complexed strongly with one mole of nickelous chloride or sulfate to give chelates that were soluble and stable in aqueous organic solvents. Preparation of the maltose and amylose complexes with bivalent metal ions provides a basis for future investigations into specific substitution reactions and fractionation of starch and starch hydrolyzates.

Absorption experiments were performed by heating a series of activated starches with 1.0 N sodium methoxide dissolved in an 80-20 mixture of tetrahydrofuran-methyl alcohol. Results showed that sodium uptake was approximately proportional to specific surface area of the starches. Previous studies of this nature conducted in methyl alcohol only failed to show any reasonable proportionality between surface area and metal uptake. This difference in results can be explained on the basis that tetrahydrofuran prevents methanol from swelling starch and changing its surface area. The reactivity of metal methoxides toward the 2-OH of activated starches decreased as the ionic radius of the metal ion increased, i.e. Li > Na > K > Ba. This research was conducted under a PL 480 grant to the Plastics Research Institute, TNO, Delft, The Netherlands.

5. Starch Reinforcement of Rubber

Purpose: The principal reinforcing agent for rubber is carbon black, which is characterized by its intense black color. Only a few expensive nonblack reinforcing agents reinforce rubber to a degree approaching carbon black. Therefore, nonblack rubbers must ordinarily be formulated with low-reinforcing fillers and have poor strength and wear properties. Recently the Northern Division discovered two new processes for reinforcement of rubber with starch. One process involves zinc starch xanthate or starch xanthide. These starch derivatives permit formulation of strong semitransparent rubbers that can be pigmented to give white or brightly colored rubber products. The second involves a crosslinked starch-resin condensation product. This product reinforces rubber as effectively as some grades of carbon black but is less expensive. Successful commercialization of these starch products as reinforcing agents for rubber would open a new market for starch potentially capable of consuming over 1 billion pounds of starch per year.

Progress: Detailed study of the starch xanthate latex masterbatching process revealed that optimum starch loadings for extrusion-processed zinc starch xanthate-SBR and starch xanthide-SBR masterbatches were 30 parts per 100 parts rubber (phr) and 45 phr, respectively. Pearl corn starch, corn flour, and wheat flours may be used interchangeably with little effect on vulcanizate properties. Moisture content at the start of extrusion processing was not an important variable in the range 10-30 percent. Additional study of starch-resin-reinforced rubbers showed that good rubber reinforcement could be obtained with only 0.03-0.06 moles of low-cost resin base per mole starch under a variety of conditions. Optimum starch or corn flour loading for oven-dried SBR masterbatches was 45 phr.

Starch-filled powdered rubber compounds have been developed. These powdered rubber compounds, like powdered plastics. can be molded or extruded without prior high-shear milling to give high-quality finished products. Industry estimates that use of powdered rubber would reduce processing costs at least 2-1/2 cents per pound of finished rubber goods by eliminating much of the heavy-duty equipment and power needed to incorporate curing and reinforcing agents, fillers, and other additives into slab rubber. In the new technology, powdered rubber is merely blended with the other ingredients and final mixing takes place during molding or extrusion. Without the addition of our starch products, rubber cannot be produced in powdered form except by costly means such as grinding in the frozen state.

In contract research at the University of Akron. Akron, Ohio, detailed studies on extrusion processing of wet masterbatches indicated conditions for complete drying by a single pass through a machine with two vacuum vents. Extrusion drying of wet starch xanthide-SBR 1500 rubber masterbatches has given starch-rubber masterbatches with vulcanizate tensile strengths greater than 2600 psi at a starch loading of 50 phr. These starch xanthide-reinforced vulcanizates have high enough tensile strength for tire treads. In these new products the reinforcing ability of starch xanthide is equal to or better than that of a wide variety of carbon blacks and most other fillers. Only the finest particle blacks and pyrogenic silicas give higher tensiles in SBR.

6. Biodegradable Surfactants and Detergent Builders

Purpose: The necessity for environmental quality control has emphasized the importance of biodegradable surfactants and has drawn attention to the probable desirability of eliminating phosphate builders from detergents. Several types of starch derivatives have chemical structures justifying evaluation of their potential as biodegradable surfactants and builders. Successful development of starch derivatives in either or both of these applications would open an important new market for starch.

Progress: Procedures were investigated for more economical preparation of carboxymethyl and dicarboxyl starch derivatives. A chemical synthesis of dialdehyde starch was developed, and both sodium chlorite and bromine oxidations of dialdehyde starch to dicarboxyl starch were carried out. Dicarboxyl starches of different carboxyl contents and molecular sizes were synthesized. Carboxymethyl starches of various degree of substitution and molecular weights were also prepared by relatively simple methods. Tests showed that several types of carboxymethyl and dicarboxyl starch were 82 to 95 percent as effective as sodium tripolyphosphate as detergent builders. Unfortunately, five-day BOD evaluations of the carboxylated starches showed that these potential builders are not readily biodegradable. Also the probability of producing starch derivatives with high carboxyl content at a cost of less than 20 cents per pound appears remote.

Eleven catalysts have been investigated for the air oxidation of starch under a grant to Western Michigan University, Kalamazoo, Michigan. They are: the ammonium salts of 6-molybdonickelate II, 9-molybdonickelate IV, 6-molybdoferrate III, 5-molybdocobaltate III, 6-molybdocobaltate III, 6-molybdochromate III; and the compounds nickel ammonium sulfate, potassium persulfate, sodium tungstate, sodium metavanadate, and potassium hexaflourotitanate. Of the six molybdenum complexes, only three gave a measurable amount of oxidation and produced starches having a blue and green color. Infrared spectra of the products indicated that nickel ammonium sulfate and potassium persulfate also oxidized starch significantly.

Under contract studies conducted at Ashland Chemical Company, Minneapolis, Minnesota, 38 surfactants remaining from a proposed list of 47 were prepared and their evaluations are nearly complete. They were made by reacting glycol or glycerol glycosides with various levels of ethylene or propylene oxides followed by esterification or etherification with long-chain aliphatic groups. One of these surfactants, a C_{12} hydroxyether derivative of ethoxylated-propoxylated glycol glycoside, showed outstanding wet-out and surface-tension-lowering characteristics and was comparable to the best commercially available surfactant. Early reports indicate that the compound has good biodegradability--a key factor in its potential applications. Two of the surfactants have been successfully prepared on a pilot-plant scale. Preliminary calculations showed that the new ether products will cost 17 cents per pound to produce and should sell for 22.5 cents per pound, a figure which would make them competitive with comparable products.

7. New Classes of High-Amylose Starch

Purpose: High-amylose corn starch is now produced commercially at a level estimated to be of the order of 30 million pounds per year. The strains of high-amylose corn used yield starch containing 50-60 and 60-70 percent apparent amylose. Corn breeders have for a number of years sought strains having increasingly high contents of amylose. More recently, however, interest has turned toward improvement of existing commercial strains; e.g., strains are desired that give higher yields per acre or that have higher total starch content. Research at the Northern Division is part of a cooperative program with corn breeders. Emphasis is placed on providing analyses of breeding strains and on development of basic information on amylose that contributes to increased industrial utilization of high-amylose starch.

Progress: The amylose analysis method was modified to permit determination of total starch in addition to amylose. This new procedure was used to analyze more than 8,000 samples in connection with the high-amylose corn development program. Over 9 percent of these showed an apparent amylose value of 85 percent or higher, a 50 percent increase over the previous year in samples of this amylose class. The highest amylose value recorded was again 90 percent. Starch contents were slightly over 70 percent in 66 samples in which the starch contained over 60 percent amylose. In 24 of these selections, the corresponding amylose content exceeded 80 percent.

X-ray studies of amylose fibers prepared from ethylenediamine have shown the existence of an ethylenediamine-amylose complex with cell dimensions similar to the V_{DMSO} amylose complex. Initial X-ray studies indicate the existence of a formamide-amylose complex in the solid state that may have significantly different cell dimensions than the V_{DMSO} complex. Total carbohydrate analysis methods have been developed in conjunction with use of a Technicon Autoanalyzer for amylose solutions in 4 M guanidine hydrochloride and in 95% DMSO - 5% H_2O. These two solvents are ones in which amylose is stable for long periods of time (retrogradation does not occur rapidly). We find that amylose is also stable in 2.5 M LiSCN over a period of about 6 months.

In research at Arizona State University, Tempe, Arizona, a V_{DMSO} type of complex previously reported has been more thoroughly examined and found to have a unit cell of 19.4 Å and, therefore, a helix diameter of 13.7 Å. Films of this complex are prepared from mixtures of DMSO and glycerine and resist conversion to other forms of amylose. New fibers have been prepared from an amylose-iodine type of complex

in which KI and I_2 are present in high concentrations. The fibers give a 3.1 Å meridional spacing which is due to iodine scattering. Zero level spacing could be indexed with a small hexagonal cell of a = b = 13.0 Å.

At the State University College of Forestry, Syracuse University, New York, quantitative evaluation of H_V light scattering envelopes from symmetric granules of potato and tapioca starch and from asymmetric granules of shoti and banana starch was accomplished. In addition, the analysis of the H_V scattering pattern from water cast amylose films was started and studies of starch gelatinization were carried out experimentally by following changes in H_V scattering patterns as a function of temperature.

Under a PL 480 grant at the University of Edinburgh, Edinburgh, Scotland, a rapid, semimicro method has been developed for estimation of starch in cereal grains. Samples of 7.5 to 20 mg. are adequate for this method which solubilizes starch with hot aqueous calcium chloride and subjects the extract to concurrent action of alpha-amylase and amyloglucosidase. The resulting glucose is quantitatively determined by use of glucose oxidase.

8. Dialdehyde Starch-Protein Plywood Glue

Purpose: Use of dialdehyde starch in plywood glue is a promising application which, if successfully commercialized, could greatly increase commercial utilization of this starch derivative.

Progress: Veneer-gluing conditions found satisfactory for fabricating interior-type southern pine plywood were used in exploratory bonding studies with yellow birch veneers and with mixtures of yellow birch and southern yellow pine veneers. The plywood obtained successfully passed all of the standard tests for interior-type plywood. A preliminary study on bonding southern pine veneers with glue containing freeze-dried chicken blood in place of soluble dried beef-pork blood appears to be very promising and could provide a profitable outlet for this waste material with concomitant reduction of water pollution in poultry processing.

In laboratory-scale veneer-gluing operations, it was found that working properties of the dialdehyde starch-protein glue used in preparing water-resistant-type plywood test panels were adversely affected by mixing the glue 24 hours in advance. Gluebond quality of test panels prepared with the 24-hour-old glue was reduced to failing levels. These adverse effects of glue aging are minimized by using a two-stage glue-mixing procedure.

9. Lower Cost Xanthan Production Process

Purpose: Commercial acceptance of xanthan gum in industrial applications has resulted in consumption of this product in significant quantities, which would be considerably increased if production costs could be decreased. Now that xanthan gum has been approved for food use by FDA, minimization of production costs assumes even greater importance. One promising approach to lowering costs is development of a continuous fermentation process for production of xanthan gum.

Progress: A single-stage continuous fermentation to produce xanthan gum has been run in the pilot plant at dilution rates of 0.15 per hour. Xanthan production rate and yield approximated values previously obtained in laboratory runs. The daily xanthan yield based on glucose consumed was 75-80 percent, and the xanthan production rate was ca. 0.7 g./hr./kg. broth. Duration of runs have not exceeded 5-7 turnovers of the fermentor contents.

Laboratory continuous fermentation studies indicate that xanthan yield and production rates are adversely affected at dilution rates greater than ca. 0.16 per hour. Batch fermentation studies on process improvements indicate that with pH control, more than 3 percent xanthan concentration can be obtained in the fermentor with a medium containing less disodium phosphate.

During the past year the Northern Division was visited by representatives of, or received inquiries from, 7 foreign and 36 domestic companies. A number of patents involving xanthan gum were issued to industrial concerns.

10. Japanese Beetle Control

Purpose: Spores of Bacillus popilliae, which cause milky disease of Japanese beetles, could serve as a selective biological insecticide harmless to man and other animals and nonproductive of environmental pollution. These spores are commercially produced from infected grubs, an expensive process that makes widespread use of spores impractical for control of Japanese beetle. The Northern Division is seeking an inexpensive, commercially feasible fermentative process for producing B. popilliae spores.

Progress: Sporulation of Bacillus popilliae NRRL B-2309S in liquid medium containing carbon now yields 3×10^7 spores per milliliter. This strain and two others also form ca. 10^5 spores per milliliter in 0.1 percent agar medium; other strains form prespores which never maturate. All strains require a labile component in yeast extract for sporulation; its nature is under investigation. Work

on metabolism of B. popilliae in diseased larvae disclosed a shift to greater pentose phosphate participation during sporulation. Tricarboxylic acid (TCA) cycle activity remains constant. Possibly the TCA cycle is not involved in sporulation because no reactions of TCA were functional in artificial culture. Lipids constitute 2.5-5.0 percent of cell dry weight in milky disease and other sporeformers associated with insects; phospholipids are predominant (55-79% to total).

In contract research at Michigan State University, East Lansing, Michigan, work is continuing on the assumption that an oxidative metabolism analogous to that in other sporeformers is required for sporulation in B. popilliae. Four variant strains were studied: Sporogenic (B-2309M), weakly sporogenic (B-2309S), asporogenic derivative of a sporogenic (B-2309N), and an asporogenic but selected strain (B-2309A). There are differences in specific metabolism, e.g., acetate oxidation, between these and wild-type strains. In addition, catalase formation is associated with sporogenicity. However, other oxidative enzymes of the tricarboxylic acid cycle and phenomena such as calcium uptake ordinarily associated with sporulation either are absent or not demonstrably related to sporogenesis in B. popilliae. The lack of correlation with acetate oxidation together with failure of acetate-utilizing strains to contain other TCA enzymes cannot be explained. Efforts to obtain sporogenic variants by chemical mutation were unsuccessful.

In grant research at Baylor University College of Medicine, Houston, Texas, serial sections of young asci of Hansenula wingei have disclosed that the appearance of four meiotic products (i.e., nuclei) takes place by lobulation and ultimate segregation of the nucleus which has undergone meiotic division. The meiotic products, together with one or more mitochondria, are then enveloped by endoplasmic reticulum to form spore anlages. It is at this stage of envelopment that impotency of some nuclei become manifest; some remain naked in the cytoplasm and never maturate. Once envelopment occurs, the anlages develop into the characteristic hat-shaped spore whose brim and crown develop as a unit.

11. L-Asparaginase Production

Purpose: Leukemia is an almost invariably fatal cancer of the tissues in the bone marrow and lymph nodes that manufacture blood cells. Recently the enzyme L-asparaginase has been shown to inhibit the growth of various lymphosarcomas in test animals and humans. Currently, the only commercial source of the enzyme is the microorganism Escherichia coli. Enzyme from more than one source is

desirable to increase the supply of enzyme, to avoid toxicity associated with E. coli enzyme, and to provide an alternate in case of allergic reaction to E. coli enzyme. The ARS Culture Collection provides an excellent opportunity to find new and superior microbial sources of L-asparaginase.

Progress: A total of 123 species of bacteria were screened for L-asparaginase production. Erwinia aroideae NRRL B-138 and Hydrogenomonas eutropha NRRL B-2804 were found to produce large quantities of the enzyme. Conditions of pH control, media selection and enrichment, and aeration were optimized to increase yields even further. Extraction procedures were investigated and lysozyme degradation was selected as the most efficient method. L-asparaginase was extracted and partially purified by column chromatography, electrophoresis, and fractional precipitation. The resulting preparation produced tumor regression in animal trials conducted by Syracuse University and by the National Cancer Institute.

The enzyme from Erwinia is reported to be far less toxic than the one from E. coli. We have been asked to prepare quantities of L-asparaginase for further testing.

12. Microbial Polysaccharides

Purpose: Colloidal substances used extensively by industry to bind water, thicken and stabilize solutions and flocculate suspensions traditionally comprise imported gums, extracts of seaplants, starches and certain starch and cellulose derivatives. Research at the Northern Division showed that polysaccharides produced by certain microorganisms could not only replace traditional materials in selected applications but also provide superior performance. One of these microbial gums, xanthan gum, is now produced commercially by several U.S. and foreign companies. To the expanding industrial market for xanthan gum can be added a large new market expected to develop now that FDA has approved xanthan for use in foods. It is anticipated that microbial sources can be found for polysaccharides that have different properties from xanthan and, therefore, that can find use in applications for which xanthan gum is unsuitable. Rising prices and limited supplies of imported gums, as well as expanding and changing demands by industry, make new and additional sources of gums necessary.

Progress: Studies relating to growth conditions of the black yeast, Y-6272, were continued during this period. Carbon sources capable of producing this polysaccharide include glucose, fructose, sucrose, and galactose; no polymer was produced with maltose or dextrins. Optimum initial pH of growth medium was 7.0. A synthetic medium

was also developed which was satisfactory for growth and polysaccharide production in reduced yields containing salts, tryptophan, asparagine, vitamins, and glucose. An improved method was developed for separation of the melanin from the microbial gum produced by Y-6272. Melanin in crude culture liquor is precipitated with dilute solution of $FeCl_2$ and mixed with EDTA (pH 7). After centrifugation, the polymer in the supernate is precipitated with alcohol. Radiochemical techniques have been applied to both components of polysaccharide Y-6272; N-acetyl glucosamine has been both identified and quantitated, and identity of N-acetyl glucosaminuronic acid has been established.

A spectrophotometric method has been established for determining hexosamines, N-acetyl hexosamines and amino acids, either in monomeric or polymeric form. The method is applicable also to the purine and pyrimidine bases of nucleic acids. Successful automation has greatly extended its potential usefulness.

The identity and origin have been established for three previously unsuspected monosaccharides in both batch and continuous fermentation liquors of polysaccharide B-1459: fructose, which arises from the glucose substrate during continuous sterilization of the media at pH 7; potassium 5-ketogluconate, which appears to be a minor metabolic product; and potassium gluconate, which occurs in very small amounts and does not seem to enter into polysaccharide production.

Growth of Sporobolomyces sp. in orthophosphate-free medium gives rise to a polymer-containing fourfold less organic phosphate and threefold more O-acetyl. D-glucose comprises 54 percent of the total hexose. Trace amounts of D-glucose were also found in polysaccharides produced by Lipomyces yeasts. Pichia sp. NRRL Y-1344 was found to produce a neutral mannan even when grown on medium containing orthophosphate. Work was completed under several PL 480 grants during the year.

At the University of Liege, Liege, Belgium, peptidoglycans of Micrococcus lysodeikticus, M. flavus, M. citreus, and Sarcina lutea were found to differ from those of other Micrococcaceae. They contain isomeric peptide dimers crosslinked directly. D-Ala-L-Ala cross-linkages predominate. Walls of M. lysodeikticus, M. flavus, and S. lutea lack teichoic acid, but contain organic P. Glycan segments consisting of 8 to 18 disaccharide units are linked through muramic acid 6-phosphate to a glucose-hexosaminuronic acid polymer.

In research at the National Sugar Institute, Kanpur, India, on the gums of 10 different Indian trees and shrubs, there have been isolated, purified, and, in most cases, identified, a total of 9 different aldobiuronic acids and 7 aldotriuronic acids in amounts ranging from 4 grams to 50 milligrams. These rare substances are composed of various combinations of uronic acids linked to neutral hexoses at carbons 2, 4, or 6.

Research at the University of Tokyo, Tokyo, Japan showed tartaric acid-producing microorganisms to be quite rare and only a few strains of Acetobacter suboxydans were isolated which produced tartaric acid. Mutation of these few strains to strains accumulating more tartrate was successful and up to about 30 percent of the glucose consumed was recovered as tartaric acid. Another acid was concurrently produced along with tartaric acid which is apparently a precursor of tartrate. This acid plus the tartaric acid would account for some 50 to 60 percent of the glucose consumed. A new method for measuring tartaric acid by polarography was developed and published. A procedure for separating tartaric acid from fermentation broths using ion-exchange methods was perfected.

C. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Mycotoxins in Grain

Purpose: Grains can be contaminated by molds capable of producing, as a result of their growth, toxic substances called mycotoxins. The possible presence of such molds and mycotoxins in grain presents a health hazard to both man and his animals. The Northern Division's research on mycotoxins is part of a broad cooperative effort involving units of the U.S. Department of Agriculture, other U.S. government agencies, various State agricultural experiment stations, and industry. Work at the Northern Division is directed towards survey of incidence of mycotoxins in cereal grains, methods of detection, and quantitative assay of grains for various mycotoxins; study of microorganisms to identify species and strains producing toxins and to determine growth conditions conducive to mycotoxin production; investigation of means for inhibiting mycotoxin production; and methods for detoxifying grain and derived foods containing aflatoxin.

Progress: Of 293 export corn samples, eight contained aflatoxin, three ochratoxin, and five zearalenone. Aflatoxin M_1 was purified in quantity. Toxin-contaminated solvent extracts of commodities were decontaminated with Flavobacterium aurantiacum. Ensiling aflatoxin-contaminated corn did not result in detoxification. A new rapid confirmatory bioassay for aflatoxin based on lysogeny was developed. Two new aflatoxin derivatives were produced and their structure determined. Manganese deficiency causes Aspergillus parasiticus to develop in a yeastlike form capable of continuous toxin synthesis.

At temperatures between -4 and +32° C. Penicillium martensii produces large amounts of penicillic acid in corn. Maximum production (8.0 g./kg. corn) occurred at 5° C. A sensitive fluorodensitometric assay was developed for this toxin. Penicillium palitans and a Cladosporium sp. produce three tremorgenic mycotoxins for which

assays have been developed. A different tremorgen is produced by Aspergillus viridenutens. Four species of Aspergillus, alliaceus, sulphureus, sclerotium, and ochraceus synthesize ochratoxin; Penicillium viridicatum is also a producer.

Contract research at South Dakota State University, Brookings, South Dakota, was completed. A total of 392 Aspergillus strains representing 153 species and varieties, were evaluated to determine the range and kind of toxicity in this genus. A total of 36 species and varieties were distinctly toxigenic. Another 45 species and varieties displayed less distinct or marginal toxicity. The results not only confirmed the toxigenicity of species and strains already known to be toxic but also, of greater importance, they revealed this property in other species and varieties not previously tested or recognized as toxic.

A consequence of the research on mycotoxins, which has major significance to human and animal health, is the clear indication of the need to routinely examine grain for a broader array of mycotoxins than heretofore was recognized.

2. Microbiology of Cereal Flours

Research on the microbiology of corn and corn flour is integrated with similar research on wheat. Results are reported under Wheat Utilization (Northern Region), Part B.

Publications and Patents

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling Artificially Dried Corn

Blinc, M., Cimerman, A., Pertot, E., and Stucin, D. (Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia). 1970. Über modifizierte maisstärke. /On modified corn starch./ Staerke 22(3), pp. 73-76.*

Brekke, O. L. 1970. Dry-milling artificially dried corn; roller-milling of degerminator stock at various moistures. Cereal Sci. Today 15(2), pp. 37-42.

Brekke, O. L., and Kwolek, W. F.[1] ([1]USDA Biometrical Services, Peoria, Illinois). 1969. Corn dry-milling: Cold-tempering and degermination of corn of various initial moisture contents. Cereal Chem. 46(5), pp. 545-559.

Cimerman, A., Blinc, M., and Stucin, D. (Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia). 1969. Beitrag zur mikroskopischen charakterisierung von hitze-modifizierten stärken. /Contribution to microscopial characterization of heat treated starches./ Staerke 21(8), pp. 211-214.*

Shelef, L., and Mohsenin, N. N. (Pennsylvania State University, University Park, Pennsylvania). 1969. Effect of moisture content on mechanical properties of shelled corn. Cereal Chem. 46(3), pp. 242-253.

Weinecke, L. A., and Montgomery, R. R. 1969. Separation of some sized and aspirated corn degerminator fractions by water flotation. Northwest. Miller 276(7), pp. 14, 16, 18.

Wolf, M. J., Cull, I. M., Helm, J. L.,[1] and Zuber, M. S.[2] ([1]Department of Agronomy, University of Missouri, Columbia, Missouri; [2]USDA Crops Research Division, University of Missouri, Columbia, Missouri). 1969. Measuring thickness of excised mature corn pericarp. Agron. J. 61, pp. 777-779.

Wolf, M. J., Khoo, U., and Seckinger, H. L. 1969. Distribution and subcellular structure of endosperm protein in varieties of ordinary and high-lysine maize. Cereal Chem. 46(3), pp. 253-263.

2. Conversion of Starch to Sugars and Sirups

Bachler, M. J., Strandberg, G. W., and Smiley, K. L. 1970. Starch conversion by immobilized glucoamylase. Biotechnol. Bioeng. 12(1), pp. 85-92.

*Research supported by PL 480 funds.

Dick, W. E., Jr., Weisleder, D., and Hodge, J. E. 1969. 2-Oxazolidinone derivatives of D-glucose and glycoaldehyde. J. Org. Chem. 34(9), pp. 2654-2660.

Fish, N. L., Mickelsen, R., Turner, M. E., Barnhart, J. L., and Cunningham, B. A. (Kansas State University, Manhattan, Kansas). 1969. Comparison of Coomassie Brilliant Blue R 250 and Amido Black 10 B for detection of whey protein bands after disc electrophoresis. J. Dairy Sci. 52(7), pp. 1095-1097.

Lehrfeld, J. 1969. A versatile and compact chromatographic chamber. J. Chem. Educ. 46(12), p. 848.

Lehrfeld, J., and Goodwin, J. C. 1969. Visualization reagents for quantitation of carbohydrates on thin-layer chromatograms by transmission densitometry. J. Chromatogr. 45(1), pp. 150-154.

Robyt, J. F., and French, D. (Iowa State University, Ames, Iowa). 1970. Multiple attack and polarity of action of porcine pancreatic α-amylase. Arch. Biochem. Biophys. 138(2), pp. 662-670.

Wolff, I. A., and Olds, D. W. Nov. 11, 1969. Levoglucosan production by pyrolysis of pretreated starches. U.S. Patent 3,478,012.

3. Low-Cost Institutional Foods from Corn

Anderson, R. A., Conway, H. F., and Peplinski, A. J. 1970. Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. Staerke 22(4), pp. 130-135.

Blessin, C. W., Deatherage, W. L., and Inglett, G. E. 1970. Chemical dehulling of dent corn. Cereal Chem. 47(3), pp. 303-308.

Cavins, J. F., and Friedman, M. 1970. An internal standard for amino acid analyses: S-β-(4-pyridylethyl)-L-cysteine. Anal. Biochem. 35(2), pp. 489-493.

Christianson, D. D. 1970. Genetic variation in cereal grains and processing effects on cereal flours as evaluated by scanning electron microscopy. Proc. Third Annu. Scanning Electron Microscope Symp., IIT Research Institute, Chicago, Illinois, April 28-30, 1970, pp. 161-168.

Christianson, D. D., Nielsen, H. C., Khoo, U., Wolf, M. J., and Wall, J. S. 1969. Isolation and chemical composition of protein bodies and matrix proteins in corn endosperm. Cereal Chem. 46(4), pp. 372-381.

Friedman, M., and Krull, L. H. 1969. A novel spectrophotometric procedure for half-cystine residues in proteins. Biochem. Biophys. Res. Commun. 37(4), pp. 630-633.

Friedman, M., and Krull, L. H. 1970. N- and C-Alkylation of peptides and proteins in dimethyl sulfoxide. Biochim. Biophys. Acta 207(2), pp. 361-363.

Gardner, H. W., Inglett, G. E., and Anderson, R. A. 1969. Inactivation of peroxidase as a function of corn processing. Cereal Chem. 46(6), pp. 626-634.

Hodge, J. E. 1969. Enzymatic browning of foods. In "Current Topics in Food and Nutrition," eds. Margaret O. Osborn and Elizabeth M. Osman, Proc. Hom. Econ. Workshop, Univ. of Iowa, Iowa City, June 10-28, 1968, pp. 127-146.

Mills, F. D., Baker, B. G., and Hodge, J. E. 1969. Amadori compounds as nonvolatile flavor precursors in processed foods. J. Agr. Food Chem. 17(4), pp. 723-727.

Mills, F. D., Weisleder, D., and Hodge, J. E. 1970. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, a novel nonenzymatic browning product. Tetrahedron Lett. (15), pp. 1243-1246.

Paulis, J. W., James, C., and Wall, J. S. 1969. Comparison of glutelin proteins in normal and high-lysine corn endosperms. J. Agr. Food Chem. 17(6), pp. 1301-1305.

Paulis, J. W., and Wall, J. S. 1969. Albumins and globulins in extracts of corn grain parts. Cereal Chem. 46(3), pp. 263-273.

4. Rennin from Microbial Sources

Ellis, J. J., and Hesseltine, C. W. 1969. Two new members of the Mucorales. Mycologia 61(5), pp. 863-872.

Hesseltine, C. W. 1969. /Fermented foods./ Shokuhin to Kagaku /Food and Science/ 12(4), pp. 1-6.

Wang, H. L., Ruttle, D. I., and Hesseltine, C. W. 1969. Antibacterial compound from a soybean product fermented by Rhizopus oligosporus. Proc. Soc. Exp. Biol. Med. 131(2), pp. 579-583.

5. Dry-Milling of High-Lysine Corn

None.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Starch Graft Copolymers

Ezra, G., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Grafting of preformed polyethylene oxide on starch. J. Appl. Polym. Sci. 13, pp. 1493-1496.*

Ezra, G., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Distribution of methoxyl groups in the methylation of starch alkoxides obtained by metallation with alkali metal naphthalene. J. Macromol. Sci.-Chem. A3(8), pp. 1589-1600.*

Fanta, G. F., Burr, R. C., Russell, C. R., and Rist, C. E. 1969. Copolymers of modified starches with polyacrylonitrile. J. Polym. Sci., Part A-1, 7(7), pp. 1675-1681.

Fanta, G. F., Burr, R. C., Russell, C. R., and Rist, C. E. 1970. Modification of wheat starch by initiating systems used for graft polymerization. Cereal Chem. 47(1), pp. 85-91.

Fanta, G. F., Burr, R. C., Russell, C. R., and Rist, C. E. 1970. Copolymers of starch and polyacrylonitrile. Influence of granule swelling on copolymer composition under various reaction conditions. J. Macromol. Sci.-Chem., A4(2), pp. 331-339.

Gugliemelli, L. A., and Weaver, M. O. Jan. 20, 1970. Process for isolating vinyl-type component of starch graft copolymers. U.S. Patent 3,491,040.

Gugliemelli, L. A., Weaver, M. O., Russell, C. R., and Rist, C. E. 1969. Base-hydrolyzed starch-polyacrylonitrile (S-PAN) graft copolymer. S-PAN-1:1, PAN M.W. 794,000. J. Appl. Polym. Sci. 13(9), pp. 2007-2017.

Reyes, Z.,[1] Clark, C. F.,[1] Comas, M.,[1] Russell, C. R., and Rist, C. E. ([1]Stanford Research Institute, Menlo Park, California). 1969. Continuous production of graft copolymers of starch with acrylamide and acrylic acid by electron preirradiation. Nucl. Appl. 6(5), pp. 509-517.

Reyes, Z.,[1] and Russell, C. R. ([1]Stanford Research Institute, Menlo Park, California). June 30, 1970. Graft polymerization of starch in novel alcohol reaction medium. U.S. Patent 3,518,176.

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*Research supported by PL 480 funds.

Sasson, S., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Anionic graft polymerization of formaldehyde on polyhydroxy polymers. Eur. Polym. J. 5, pp. 315-318.*

Sasson, S., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Anionic graft polymerization of 4-vinyl pyridine on starch and dextrin. Eur. Polym. J. 5, pp. 369-378.*

Tahan, M., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Alkali metal alkoxide derivatives of starch and dextrin as initiators of graft polymerization of methacrylonitrile. Eur. Polym. J. 5, pp. 347-359.*

Tahan, M., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Anionic graft polymerization of methacrylonitrile on potassium starch alkoxide. J. Polym. Sci., Part A-1, 7(7), pp. 1839-1860.*

Tahan, M., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Anionic graft polymerization of ethylene oxide on starch. I. J. Polym. Sci., Part A-1, 7(7), pp. 1815-1824.*

Tahan, M., and Zilkha, A. (The Hebrew University, Jerusalem, Israel). 1969. Anionic graft polymerization of ethylene oxide on starch. II. Structure of the graft polymers. J. Polym. Sci., Part A-1, 7(7), pp. 1825-1837.*

2. Starch Xanthides as Wet-Strength Agents in Paper

Albano, E. L., Horton, D., and Lauterbach, J. H. (The Ohio State University, Columbus, Ohio). 1968. Addition reactions of a 2,3-unsaturated sugar. Chem. Commun. (7), pp. 357-358.

Bell, R. H., Horton, D., and Williams, D. M. (The Ohio State University, Columbus, Ohio). 1968. Photolysis of dimethylthiocarbamates as a route to deoxy-sugars. Chem. Commun. (6), pp. 323-324.

Clode, D. M., and Horton, D. (The Ohio State University, Columbus, Ohio). 1970. Synthesis of the 6-aldehydo derivative of cellulose, and a mass-spectrometric method for determining degree of substitution. Carbohyd. Res. 12(3), pp. 477-479.

Clode, D. M., Horton, D., Meshreki, M. H., and Shoji, H. (The Ohio State University, Columbus, Ohio). 1969. Linear polyaldehydes and polyalkenes derived from starch. Chem. Commun. (13), pp. 694-695.

*Research supported by PL 480 funds.

Doane, W. M., Shasha, B. S., Stout, E. I., Russell, C. R., and Rist, C. E. 1969. Ring-opening reactions of trans-carbonates and thionocarbonates. Carbohyd. Res. 11(3), pp. 321-329.

Horton, D., and Pardoe, W. D. (The Ohio State University, Columbus, Ohio). 1970. Calculation of degree of substitution of polysaccharides: A Fortran computer program. Carbohyd. Res. 12(2), pp. 269-272.

Schurz, J., Schmidt, K. H., Uragg, H., and Renger, J. (University of Graz, Graz, Austria). 1969. Rheologische charakterisierung von stärkepasten und streichfarben. /Rheological characterization of starch-pastes and coating formulations./ Fortdrucke 23(10A), pp. 784-794.*

Shasha, B. S. 1969. Determination of starch component of starch xanthide in paper. Tappi 52(12), pp. 2287-2288.

Shasha, B. S., Doane, W. M., Russell, C. R., and Rist, C. E. 1969. Reactions of chlorine with some thiocarbonyl sugar derivatives. J. Org. Chem. 34(6), pp. 1642-1645.

Shasha, B. S., Doane, W. M., Russell, C. R., and Rist, C. E. 1969. Unsymmetrical dithiobis(thioformates). Carbohyd. Res. 10(3), pp. 449-455.

Shasha, B. S., Doane, W. M., Russell, C. R., and Rist, C. E. 1970. A sulfenyl iodide in the mixture formed by oxidation of a sugar xanthate with iodine. Carbohyd. Res. 13(3), pp. 457-460.

Smith, H. E., Katz, H. C., Gordon, S. H., Russell, C. R., and Rist, C. E. 1969. Oxidative coupling of starch xanthate with chlorine. Staerke 21(9), pp. 225-228.

Trimnell, D., Doane, W. M., Russell, C. R., and Rist, C. E. 1969. Migration of thiolthiocarbonyl groups in selectively protected methyl α-D-glucopyranoside xanthates. Carbohyd. Res. 11(4), pp. 497-507.

Trimnell, D., Doane, W. M., Russell, C. R., and Rist, C. E. 1970. Methyl 2,3-di-O-α-D-glucopyranoside 4,6-carbonate. Carbohyd. Res. 13(2), pp. 301-305.

Wing, R. E., Doane, W. M., and Rist, C. E. 1970. Sugars with reactive substituents: Preparation of 3-O-(2,3-epoxypropyl and 2,3-epithiopropyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose. Carbohyd. Res. 12(2), pp. 285-289.

*Research supported by PL 480 funds.

Wing, R. E., Doane, W. M., and Rist, C. E. 1970. Oxirane ring-opening reactions of 3-O-(2,3-epoxypropyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose. Carbohyd. Res. 12(3), pp. 347-353.

3. Cationic Starches and Flours for Paper

Cramer, W. D.,[1], Hanson, T. P.,[1] Tsao, G. T.,[1] and Lancaster, E. B. ([1]Iowa State University, Ames, Iowa). 1969. Water vapor sorption in starch granules. J. Macromol. Sci.-Phys. B3(4), pp. 611-622.

Grob, V. D., Squires, T. G., and Vercellotti, J. R. (University of Tennessee, Knoxville, Tennessee). 1969. Glycosyl chlorides through reaction with zinc chloride-thionyl chloride. Carbohyd. Res. 10(4), pp. 595-597.

Heath, H. D., Ernst, A. J., Hofreiter, B. T., and Rankin, J. C. 1969. Cationic aminoethyl cereal flours as wet-end paper additives. Tappi 52(9), pp. 1647-1651.

Roth, D. D.,[1] Tsao, G. T.,[1] and Lancaster, E. B. ([1]Iowa State University, Ames, Iowa). 1970. Thermal conductivity of starch granules. Staerke 22(2), pp. 40-44.

Smith, H. E., Russell, C. R., Holzapfel, M. M., and Rist, C. E. 1969. Sulfated wheat flours of low sulfur content. Northwest. Miller 276(4), pp. 13-15.

Vercellotti, J. R., Nienaber, N., and Chang, C. J. Marquette University, Milwaukee, Wisconsin). 1970. Monosaccharides as O-glycosyl leaving groups from 3-hydroxy amino acids during base-catalyzed elimination. Carbohyd. Res. 13(1), pp. 63-74.

Wheelock, T. D.,[1] and Lancaster, E. B. ([1]Iowa State University, Ames, Iowa). 1970. Thermal properties of wheat flour. Staerke 22(2), pp. 44-48.

4. Starch-Derived Chemicals for Plastics and Coatings

Black, W. A., Dewar, E. T., and Rutherford, D. (A. D. Little Research Institute, Musselburgh, Scotland). Aug. 26, 1969. 1,6-Diamino-2,3:4,5-di-O-benzilidine-1,6-dideoxygalactitol. U.S. Patent 3,463,790.*

*Research supported by PL 480 funds.

Lakshmanan, C. M., Gal-Or, B., and Hoelscher, H. E. (University of Pittsburgh, Pittsburgh, Pennsylvania). 1969. Production of levoglucosan by pyrolysis of carbohydrates. Ind. Eng. Chem., Prod. Res. Develop. 8(3), pp. 261-267.

McGuire, T. A., and Mehltretter, C. L. 1969. Preparation of (carboxymethyl)-cyclohexyldimethylammonium chloride hydrazide. J. Med. Chem. 12(4), p. 747.

McKillip, W. J.,[1] Kellen, J. N.,[1] Impola, C. N.,[1] Buckney, R.W.,[1] and Otey, F. H. ([1]Ashland Chemical Company, Bloomington, Minnesota). 1970. Glycol glycosides in alkyds. J. Paint Technol. 42(544), pp. 312-319.

Maher, G. G. Apr. 1, 1969. Starch polyethyleniminothiourethane additives for paper. U.S. Patent 3,436,305.

Mehltretter, C. L. Nov. 11, 1969. Hydrazides of fatty quaternary ammonium salts of glycine as retention agents. U.S. Patent 3,477,904.

Mehltretter, C. L. Mar. 10, 1970. Azo dyes of starch anthranilates and process of preparing same. U.S. Patent 3,499,886.

Mehltretter, C. L., McGuire, T. A., Glass, C. A., and Wilham, C. A. 1969. Effect of temperature and concentration on the chlorohydrination of allyltrimethylammonium chloride. Ind. Eng. Chem. Prod. Res. Develop. 8(3), pp. 279-281.

Mehltretter, C. L., and Wilham, C. A. June 2, 1970. Hypochlorite modified cyanoethylated starch having intact granule form. U.S. Patent 3,515,718.

Nayak, U. G., and Whistler, R. L. (Purdue University, Lafayette, Indiana). 1969. Nucleophilic displacement in 1,2:5,6-di-O-isopropylidene-3-O-(p-tolylsulfonyl)-α-D-glucofuranose. J. Org. Chem. 34(12), pp. 3819-3822.

Otey, F. H., Westhoff, R. P., Kwolek, W. F.,[1] Mehltretter, C. L., and Rist, C. E. ([1]USDA Biometrical Services, Peoria, Illinois). 1969. Urethane plastics based on starch-derived glycosides. Ind. Eng. Chem., Prod. Res. Develop. 8(3), pp. 267-274.

Sinclair, H. B. 1969. Di-O-bromoethylidene-D-mannitol formation. J. Org. Chem. 34(12), pp. 3845-3848.

Sinclair, H. B. 1970. 1,3:4,6-Di-O-benzylidene-D-mannitol. Carbohyd. Res. 12(1), pp. 150-152.

Sinclair, H. B., and Sleeter, R. T. 1970. Anomeric configuration in carbohydrates. Tetrahedron Lett. (11), pp. 833-836.

Srivastava, H. C., Ramalingam, K. V., and Chaudhari, A. S. (Ahmedabad Textile Industry's Research Association, Ahmedabad, India). 1969. Synthesis of 1,2-O-ethylene-D-glucofuranose. Tetrahedron Lett. (31), pp. 2643-2646.*

Srivastava, H. C., Ramalingam, K. V., Doshi, N. M., and Chaudhari, A. S. (Ahmedabad Textile Industry's Research Association, Ahmedabad, India). 1970. The isolation of 1,2-O-ethylene derivatives of D-glucose from O-(2-hydroxyethyl) starch. Carbohyd. Res. 12(1), pp. 23-28.*

Srivastava, H. C., Singh, P. P., and Dave, G. B. (Ahmedabad Textile Industry's Research Association, Ahmedabad, India). 1969. Codextrinization of starch and gum karaya. Part I. Fractionation and chemical examination of the codextrin. Staerke 21(5), pp. 123-127.*

Whistler, R. L., and Anisuzzaman, A. K. M. (Purdue University, Lafayette, Indiana). 1969. Photolysis of methyl 2,3,4-tri-O-acetyl-6-azide-6-deoxy-α-D-glucopyranoside. J. Org. Chem. 34(12), pp. 3823-3824.

Wing, R. E., and BeMiller, J. N. (Southern Illinois University, Carbondale, Illinois). 1969. Methyl terminal-4-deoxy-, terminal-4-O-ethyl-, and terminal -4-O-butyl-malto-oligosaccharides: Synthesis, and hydrolysis with beta-amylase. Carbohyd. Res. 10(3), pp. 371-377.

Wing, R. E., and BeMiller, J. N. (Southern Illinois University, Carbondale, Illinois). 1969. Preparation of alkyl α-D-glucopyranosides. Carbohyd. Res. 10(3), pp. 441-448.

Wolfrom, M. L., and Bhattacharjee, S. S. (The Ohio State University, Columbus, Ohio). 1969. Starch acetals: Acid sensitivity and preferred site of reaction. Staerke 21(5), pp. 116-118.

Wolfrom, M. L., Bhattacharjee, S. S., and Lederkremer, R. M. de (The Ohio State University, Columbus, Ohio). 1969. Reaction of carbohydrates with vinyl ethers; a differential hydrolysis. Carbohyd. Res. 11(1), pp. 148-150.

*Research supported by PL 480 funds.

Wolfrom, M. L., and Parekh, G. G. (The Ohio State University, Columbus, Ohio). 1969. Reaction of alkyl vinyl ethers with D-galactose diethyl dithioacetal. Carbohyd. Res. 11(4), pp. 547-557.

5. Starch Reinforcement of Rubber

Buchanan, R. A., and Russell, C. R. Nov. 25, 1969. Starch resin reinforced rubbers. U.S. Patent 3,480,572.

Buchanan, R. A., Russell, C. R., and Weislogel, O. E. May 6, 1969. Reinforced rubber by coprecipitation with starch xanthate or pregelatinized starch. U.S. Patent 3,442,832.

Stephens, H. L., Murphy, R. J., and Reed, T. F. (Institute of Polymer Science, University of Akron, Akron, Ohio). 1969. The use of starch as a reinforcing filler for rubber. Rubber World 161(2), pp. 77-81.

6. Biodegradable Surfactants and Detergent Builders

Otten, J. G., and Mehltretter, C. L. 1970. Separation of cis- and trans-aconitic acids by thin-layer chromatography. Anal. Biochem. 35(1), pp. 302-303.

7. New Classes of High-Amylose Starch

Adkins, G. K., and Greenwood, C. T. (University of Edinburgh, Edinburgh, Scotland). 1969. Studies of starches of high amylose content. Part X. An improved method for the fractionation of maize and amylomaize starches by complex formation from aqueous dispersion after pretreatment with methyl sulphoxide. Carbohyd. Res. 11(2), pp. 217-224.*

Banks, W., and Greenwood, C. T. (University of Edinburgh, Edinburgh, Scotland). 1969. The application of the Ptitsyn Theory to viscosity data for amylose and amylose acetate in various solvents. In "Polymer. The Science and Technology of Polymers and Biopolymers," vol. 10, pp. 257-264. London.*

Banks, W., and Greenwood, C. T. (University of Edinburgh, Edinburgh, Scotland). 1969. Studies on starch-degrading enzymes. Part XI. The stability of crystalline sweet potato beta-amylase in aqueous solution. Staerke 21(7), pp. 177-180.*

*Research supported by PL 480 funds.

Banks, W., and Greenwood, C. T. (University of Edinburgh, Edinburgh, Scotland). 1970. Comments on "Enzymic methods for the microdetermination of glycogen and amylopectin, and their unit chain-lengths." Arch. Biochem. Biophys. 136(1), pp. 320-322.*

Banks, W., Greenwood, C. T., and Muir, D. D. (University of Edinburgh, Edinburgh, Scotland). 1970. The characterization of starch and its components. Part 2. The semi-micro estimation of the starch content of cereal grains and related materials. Staerke 22(4), pp. 105-108.*

Banks, W., Greenwood, C. T., and Sloss, J. (University of Edinburgh, Edinburgh, Scotland). 1969. Light-scattering studies on aqueous solutions of amylose and amylopectin. Carbohyd. Res. 11(3), pp. 399-406.*

Blackwell, J., Sarko, A., and Marchessault, R. H. (State University College of Forestry, Syracuse, New York). 1969. Chain conformation in B-amylose. J. Mol. Biol. 42(2), pp. 379-383.

Borch, J., Sarko, A., and Marchessault, R. H. (State University College of Forestry, Syracuse, New York). 1969. Anisotropic scattering by single starch granules. Staerke 21(11), pp. 279-284.

Dintzis, F. R., and Tobin, R. 1969. Optical rotation of some α-1,4-linked glucopyranosides in the system H_2O-DMSO and solution conformation of amylose. Biopolymers 7(4), pp. 581-583.

Mark, A. M., and Mehltretter, C. L. 1969. Water-soluble films from partially acetylated high-amylose corn starch. Staerke 21(4), pp. 92-96.

Mark, A. M., and Mehltretter, C. L. 1970. Acetylation of high-amylose corn starch. Influence of pretreatment techniques on reaction rate and triacetate solubility. Staerke 22(4), pp. 108-110.

Panzica, R. P., Yuen, G. U., and Zaslow, B. (Arizona State University, Tempe, Arizona). 1969. Reactivity of "V" amylose towards hydrogen chloride. Carbohyd. Res. 10(3), pp. 343-349.

Purvinas, R. M., and Zobel, H. F. 1969. Optical rotatory dispersion of amylose films. Carbohyd. Res. 10(1), pp. 129-139.

*Research supported by PL 480 funds.

Roth, W. B., and Mehltretter, C. L. 1970. Films from mixtures of viscose and alkali high-amylose corn starch. J. Appl. Polym. Sci. 14(5), pp. 1387-1389.

8. Dialdehyde Starch-Protein Plywood Glue

None.

9. Lower Cost Xanthan Production Process

Dintzis, F. R., Babcock, G. E., and Tobin, R. 1970. Studies on dilute solutions and dispersions of the polysaccharide from Xanthomonas campestris NRRL B-1459. Carbohyd. Res. 13(2), pp. 257-267.

Rogovin, S. P. Dec. 23, 1969. Continuous process for producing Xanthomonas heteropolysaccharide. U.S. Patent 3,485,719.

Silman, R. W., and Rogovin, P. 1970. Continuous fermentation to produce xanthan biopolymer: Laboratory investigation. Biotechnol. Bioeng. 12(1), pp. 75-83.

10. Japanese Beetle Control

Bennett, G. A., and Shotwell, O. L. 1970. A lipoglycoprotein from hemolymph of healthy and diseased larvae of the Japanese beetle, Popillia japonica. J. Invertebr. Pathol. 15(2), pp. 157-164.

Bulla, L. A., St. Julian, G., Rhodes, R. A., and Hesseltine, C. W. 1969. Scanning electron and phase-contrast microscopy of bacterial spores. Appl. Microbiol. 18(3), pp. 490-495.

Ellis, J. J., Bulla, L. A., St. Julian, G., and Hesseltine, C. W. 1970. Scanning electron microscopy of fungal and bacterial spores. Proc. Third. Annu. Scanning Electron Microscope Symp., IIT Research Institute, Chicago, Illinois, April 28-30, 1970, pp. 145-152.

Hrubant, G. R., and Rhodes, R. A. 1969. Enzymatic lysis of vegetative cells of Bacillus popilliae and other sporeformers. Can J. Microbiol. 15(8), pp. 827-833.

St. Julian, G., Jr. Aug. 26, 1969. Process for delayed production of Japanese beetle larvae. U.S. Patent 3,463, 122.

Schwartz, P. H., Jr.,[1] and Sharpe, E. ([1]USDA Entomology Research Division, Moorestown, New Jersey). 1970. Infectivity of spores of Bacillus popilliae produced on a laboratory medium. J. Invertebr. Pathol. 15(1), pp. 126-128.

Sharpe, E. S., St. Julian, G., and Crowell, C. 1970. Characteristics of a new strain of Bacillus popilliae sporogenic in vitro. Appl. Microbiol. 19(4), pp. 681-688.

Weiner, B. A. Nov. 4, 1969. Polarographic electrode structure. U.S. Patent 3,476,670.

11. L-Asparaginase Production

Ciegler, A., and Lindenfelser, L. A. 1969. An aintbiotic complex from Alternaria brassicicola. Experientia 25(7), pp. 719-720.

Peterson, R. E., and Ciegler, A. 1969. L-Asparaginase production by various bacteria. Appl. Microbiol. 17(6), pp. 929-930.

Peterson, R. E., and Ciegler, A. 1969. L-Asparaginase production by Erwinia aroideae. Appl. Microbiol. 18(1), pp. 64-67.

12. Microbial Polysaccharides

Bajpai, K. S., Chandrasekharan, V., Mukherjee, S., and Shrivastava, A. N. (National Sugar Institute, Kanpur, India). 1970. Isolation of pure aldobiouronic and aldotriouronic acids from plant gums and mucilages. Indian J. Chem. 8(1), pp. 48-50.*

Ciegler, A. 1969. Microbiological transformation of terpenes. In "Fermentation Advances," ed. D. Perlman, pp. 689-714. New York.

Campbell, J. N., Leyh-Bouille, M., and Ghuysen, J.-M. (University of Liege, Liege, Belgium). 1969. Characterization of the Micrococcus lysodeikticus type of peptidoglycan in walls of other Micrococcaceae. Biochemistry 8(1), pp. 193-200.*

Davis, E. N., Wallen, L. L., Goodwin, J. C., Rohwedder, W. K., and Rhodes, R. A. 1969. Microbial hydration of cis-9-alkenoic acids. Lipids 4(5), pp. 356-362.

Ghuysen, J.-M., Dierickx, L., Coyette, J., Leyh-Bouille, M., Guinand, M., and Campbell, J. N. (University of Liege, Liege, Belgium). 1969. An improved technique for the preparation of Streptomyces peptidases and N-acetylmuramyl-L-alanine amidase active on bacterial wall peptidoglycans. Biochemistry 8(1), pp. 213-222.*

*Research supported by PL 480 funds.

Guinand, M., Ghuysen, J.-M., Schleifer, K. H., and Kandler, O. (University of Liege, Liege, Belgium). 1969. The peptidoglycan in walls of Butyribacterium rettgeri. Biochemistry 8(1), pp. 200-207.*

Herman, A. I. 1970. Variability in the yeast Hansenula holstii. Amer. J. Bot. 57(3), pp. 304-308.

Herman, A. I. 1970. A sex-specific growth response in the yeast Kluyveromyces lactis. Can. J. Genet. Cytol. 12(1), pp. 66-69.

Heijenoort, J. van, Elbaz, L., Dezelee, P., Petit, J.-F., Bricas, E., and Ghuysen, F.-M. (University of Liege, Liege, Belgium). 1969. Structure of the meso-diaminopimelic acid containing peptidoglycans in Escherichia coli B and Bacillus megaterium KM. Biochemistry 8(1), pp. 207-213.*

Martignoni, M. E.,[1] Iwai, P. J.,[1] and Wickerham, L. J. ([1] USDA Forestry Sciences Laboratory, Corvallis, Oregon). 1969. A candidiasis in larvae of the Douglas-fir tussock moth, Hemerocampa pseudotsugata. J. Inverterb. Pathol. 14(1), pp. 108-110.

Slodki, M. E., Safranski, M. J., Hensley, D. E., and Babcock, G. E. 1970. Variation in composition of yeast phosphohexosans. Appl. Microbiol. 19(6), pp. 1019-1020.

Spencer, J. F. T.,[1] Gorin, P. A. J.,[1] and Wickerham, L. J. ([1]Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan). 1970. Proton magnetic resonance spectra of the mannans of some new species of Hansenula and their phylogenetic significance. Can. J. Microbiol. 16(6), pp. 445-448.

Takagi, M., and Ono, S. (University of Osaka Prefecture, Sakai, Japan). 1969. Polarographic studies on quinoxalines for the determination of carbonyl groups in starches. Bull. Univ. Osaka Pref., Ser. B, 21, pp. 77-122.

Wickerham, L. J., Kurtzman, C. P., and Herman, A. I. 1970. Sexual reproduction in Candida lipolytica. Science 167(3921), p. 1141.

Yamada, K., Minoda, Y., Kodama, T., and Kotera, U. (University of Tokyo, Tokyo, Japan). 1969. Microbial formation of tartaric acid from glucose. In "Fermentation Advances," ed. D. Perlman, pp. 541-560. New York.*

Slodki, M. E., and Boundy, J. A. 1970. Yeast phosphohexosans. Develop. Ind. Microbiol. 11, pp. 86-91.

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*Research supported by PL 480 funds.

C. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Mycotoxins in Grain

Ciegler, A. 1969. Tremorgenic toxin from Penicillium palitans. Appl. Microbiol. 18(1), pp. 128-129.

Detroy, R. W., and Hesseltine, C. W. 1969. Transformation of aflatoxin B_1 by steroid hydroxylating fungi. Can. J. Microbiol. 15(6), pp. 495-500.

Ellis, J. J. 1969. An orange-yellow mutant of Aspergillus parasiticus produces aflatoxin. Mycologia 61(3), pp. 651-653.

Hesseltine, C. W. 1969. Mycotoxins. Mycopathol. Mycol. Appl. 39(3-4), pp. 371-383.

Hesseltine, C. W., Sorenson, W. G., and Smith, M. 1970. Taxonomic studies of the aflatoxin-producing strains in the Aspergillus flavus group. Mycologia 62(1), pp. 123-132.

Kaneshiro, T., and Thomas, P. J. 1969. Methylation of fatty acids in a methionine-dependent Agrobacterium tumefaciens controlled with exogenous methionine. Biochim. Biophys. Acta 187(1), pp. 26-35.

Lai, M.,[1] Semeniuk, G.,[1] and Hesseltine, C. W. ([1]South Dakota State University, Brookings, South Dakota). 1970. Conditions for production of ochratoxin A by Aspergillus species in a synthetic medium. Appl. Microbiol. 19(3), pp. 542-544.

Lillehoj, E. B., and Ciegler, A. 1969. Biological activity of aflatoxin B_{2a}. Appl. Microbiol. 17(4), pp. 516-519.

Lillehoj, E. B., Ciegler, A., and Detroy, R. W. 1970. Fungal toxins. In "Essays in Toxicology," ed. Frank R. Blood, vol. 2, chap. 1, pp. 1-136. New York

Shotwell, O. L., Hesseltine, C. W., Burmeister, H. R., Kwolek, W. F.,[1] Shannon, G. M., and Hall, H. H. ([1]USDA Biometrical Services, Peoria, Illinois). 1969. Survey of cereal grains and soybeans for the presence of aflatoxin: I. Wheat, grain sorghum, and oats. Cereal Chem. 46(5), pp. 446-454.

Shotwell, O. L., Hesseltine, C. W., Burmeister, H. R., Kwolek, W. F.,[1] Shannon, G. M., and Hall, H. H. ([1]USDA Biometrical Services, Peoria, Illinois). 1969. Survey of cereal grains and soybeans for the presence of aflatoxin. II. Corn and soybeans. Cereal Chem. 46(5), pp. 454-463.

Shotwell, O. L., Hesseltine, C. W., and Goulden, M. L. 1969. Ochratoxin A: Occurrence as natural contaminant of a corn sample. Appl. Microbiol. 17(5), pp. 765-766.

Stubblefield, R. D., Shannon, G. M., and Shotwell, O. L. 1969. Aflatoxins: Improved resolution by thin layer chromatography. J. Ass. Off. Anal. Chem. 52(4), pp. 669-672.

Stubblefield, R. D., Shotwell, O. L., Shannon, G. M., Weisleder, D., and Rohwedder, W. K. 1970. Parasiticol: A New metabolite from Aspergillus parasiticus. J. Agr. Food Chem. 18(3), pp. 391-393.

Vesonder, R. F., and Stodola, F. H. 1969. Formation of a C_{22} dihydroxyketo acid by a yeast. Can. J. Chem. 47(7), pp. 1247-1249.

2. Microbiology of Cereal Flours

Publications are reported under Wheat Utilization (Northern Region), Section B.

WHEAT UTILIZATION (NORTHERN REGION)

Problems and Objectives

The dominant factor in the wheat economy of the United States continues to be a production capacity that can outpace U.S. and foreign demand. Research on wheat seeks to solve the problems hindering the development of markets for the full productive capacity of U.S. agriculture. The Northern Division research seeks to improve yield and quality of wheat flour for use in foods and to find new industrial markets for wheat, particularly in the Pacific Northwest where corn is not grown.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Improve yield and quality of wheat milling fractions.

2. Develop information on microorganisms found in wheat and wheat products.

3. Convert wheat flour and starch to chemical products whose use will be economically advantageous to selected segments of the paper industry.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling and Fractionation

Purpose: The process of milling wheat has changed very little since the turn of the century. New approaches to milling and processing could improve economics or extend food utilization by providing wheat fractions with higher quality or specialized properties for specific food applications. Basic to research on milling and fractionation are studies on the microstructure of the wheat kernel and on the chemical and physical properties of wheat proteins, which are responsible for the unique ability of wheat flour to form elastic doughs. Research on wheat at the Northern Division is coordinated with research on food utilization of wheat conducted at the Western Division.

Progress: Fractions of reduced fiber content for potential use in foods were produced from defatted wheat or corn germ by screening, fine grinding, and air classification. For a crude fiber level

reduced to 1.5 percent, product yields varied between 45 and 70 percent when processing wheat germ stock (3.0% fiber initially) and 40-55 percent for corn germ stock (4.4% fiber initially), depending upon processing procedure used. Protein contents of products were comparable to that of starting materials (24-28% range). Ash content of product was unchanged for wheat but increased for the corn germ fraction.

Direct steaming of wheat has been shown to harden the kernel with respect to its ability to resist deformation. However, a direct steaming of durum wheat pretempered to 25 percent moisture resulted in a lower yield of semolina when the wheat was milled (68% vs. 79% for control) and the product contained more of the undesirable bran specks. Steam treatment had no effect on degree of protein shift during subsequent grinding and air classification of the semolina flour.

Extraction of wheat flour with urea solutions removes gliadin proteins and some low molecular weight (MW) glutenin proteins, but nitrogenous materials remain in the extracted residue. The unextracted protein, which can be removed with phenol-acetic acid-water or by extraction with acetic acid after reduction and alkylation, behaves like glutenin upon gel filtration or starch gel electrophoresis. Sonic oscillation dissociates isolated glutenin sufficiently to allow fractionation by gel filtration on agarose. Lower MW fractions migrate in bands like slow-moving gliadin proteins during gel electrophoresis. Other fractions give poorly defined electrophoresis patterns. The glutenin fractions differ in amino acid composition and enzymic digestion pattern. Such results implicate associative forces and unique peptide structures as determinants of glutenin's cohesive properties. The unusual nature of glutenin is also evidenced by comparison of fragments from gluten proteins. Peptides from glutenin are larger than those from gliadin even after digestion with pepsin and Pronase. The size difference involved ninhydrin-negative peptides that contain sulfur amino acids. Digests of glutenin proteins from different wheat varieties differ in type and amount of high MW fragments.

Conformations of gluten from seven varieties of wheat were studied by optical rotatory dispersion in 0.002 N HCl. Alpha-helix contents calculated from Moffitt equation indicated no significant difference between the seven varieties. In addition, two varieties each of glutenin and gliadin were studied in two solvents by the same method; no varietal difference was found when helix contents were compared. An adequate amount of pure gamma-1 gliadin was prepared by column chromatography for characterization study. Conformations of gluten, bovine serum albumin, and reduced-alkylated gluten and bovine serum

albumin were studied by infrared spectra. The results indicated that alpha-helix and unordered structure are present in each protein whereas beta structure appears to be absent.

Endosperm protein was dispersed on a clean water surface and stained either positively or negatively with heavy metal salts. Sodium phosphotungstate, commonly used as a protein negative stain, failed to reveal the thin protein strands present in positively stained preparations. However, typical negative stained particles were visible in many areas. These particles resemble in size and shape particles produced from a surface dispersion of a purified gliadin sample. Measurements of particles showed that those from the hard wheats averaged 260 Å diameter, whereas those from the soft wheats averaged only 175 Å diameter. Gliadin particles averaged 155 Å diameter.

Under a PL 480 grant to the Agricultural University of Poznan, Poznan, Poland, a method of kernel sectioning was devised and standardized for routine use. Histochemical reactions have been explored for esterase activity, sulfhydryl groups, amino groups, basic proteins and the amino acids tyrosine and tryptophane. Activities were localized in different parts of the kernel: esterase in the aleurone layer and sulfhydryl in both aleurone and embryo. After reduction of the sections with potassium cyanide, disulfide was detected also in the endosperm. Amino groups occurred in all parts of the kernel including proteins of the starchy endosperm. Basic proteins containing lysine occurred in the embryo, and those with both lysine and arginine in the aleurone layer. A positive reaction for tryptophane was reported in the embryo, aleurone, and also in the starchy endosperm.

In Buhler mill streams, highest proteolytic activity was in the bran and shorts. In general, proteolytic activity varied directly with the protein content of the mill stream. Disulfide content showed a negative correlation with protein content of the milled fraction. Proteolytic activity of air-classified bran and shorts fractions dropped markedly.

B. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Microbiology of Cereal Flours

Purpose: Wheat flour normally contains a variable number of microorganisms. Some of these originate in or on the wheat grain, but others enter the flour during milling. Unless flour is stored under damp conditions, these microorganisms cause no problems. They are destroyed when flour is made into bread or other baked goods.

During recent years, however, use of refrigerated dough products has shown steady growth. Since these products are moist, microorganisms can grow and eventually cause spoilage, a major problem confronting the refrigerated dough industry. The microbiology of corn and corn flour is growing in importance because of increasing use of corn flour in a variety of refrigerated doughs and pastries and in various types of convenience mixes. Spoilage of moist products containing corn starch, e.g. mayonnaise and salad dressing, also poses a significant problem to the food industry. For obvious scientific reasons, it is desirable to investigate microbiology of cereal flours as an integrated program covering both wheat and corn.

Progress: Work was concluded on the microbiology of refrigerated dough products. Exploratory work on the microbial spoilage of mayonnaise and salad dressing has shown only a few species of bacteria and a yeast (Saccharomyces sp.) to be responsible for spoilage. Studies on low-temperature storage of high-moisture corn have shown Penicillia and Fusaria to predominate after 6 months. Penicillium martensii became the predominant species after storage and produced considerable amounts of the mycotoxin penicillic acid. This finding indicates a potential hazard exists when high-moisture corn is stored at low temperature.

The ARS Culture Collection played a role in research on microbiology of cereals by serving as a source of cultures for comparative purposes and by providing taxonomic expertise. In addition, the Collection continues to serve the important functions of supplying cultures to a broad segment of the scientific community and in serving as a research group carrying out microbiological research of fundamental and practical import to the missions of ARS.

As of January 1, 1970, the ARS Culture Collection maintained a total of 40,239 strains of molds, yeasts, bacteria and actinomycetes. The permanent collection contained 13,142 of these strains. The remainder are in temporary collections. During 1969, the Collection distributed 3,142 cultures of which 1,552 were sent to investigators in the United States and 1,590 were sent abroad. New major additions to the Collection during 1969 included more than 100 gymnoascaceous molds, 20 molds isolated from copper mines, 26 toxin-producing strains in the Aspergillus flavus group, 422 isolates of mucoraceous molds from corn, 15 entomogenous bacteria, 7 Zoogloeas, 35 strains of streptomycetes isolated from tobacco and 7 actinomycetes isolated from stored wood chips.

Research on microbiology of cereals was supported by several grants under PL 480. At the University of Allahabad, Allahabad, India, it was observed that washing of wheat prior to milling actually increased

the number of bacteria over the unwashed wheat. Unlike the U.S., in India the dominant mold species in wheat flour belong to the genus Aspergillus. This may reflect a difference in storage conditions and the higher temperatures. Besides Aspergillus, Penicillium, Alternaria, Absidia, Cladosporium, Cunninghamella, Fusarium, Rhizopus, and Mucor are the common genera of molds present in Indian cereals. Actinomycetes were found to be present in numbers of a few hundred per gram of wheat and wheat flour.

A total of 61 streptomycete cultures were received during the year from the National Institute of Agronomic Investigations, Madrid, Spain. Accompanying the cultures were supporting cultural data and electron micrographs for each strain. Routine shaken-flask screening tests at the Northern Division indicated that 48 of the streptomycetes produced antibiotics under the test conditions. Thirty-five of the antibiotics demonstrated activity against a gram-positive bacterium (Bacillus subtilis), 4 against a gram-negative bacterial plant pathogen (Agrobacterium tumefaciens), 39 against a mold (Mucor ramannianus), and 24 against a yeast (Saccharomyces pastorianus). This work has now been completed.

The Central Drug Research Institute, Lucknow, India, supplied a total of 15 streptomycete cultures along with supporting cultural data. Routine shaken-flask screening tests indicated that 11 of the 15 strains produced antibiotics. Ten demonstrated activity against a gram-positive bacterium (Bacillus subtilis), 5 against a gram-negative bacterial plant pathogen (Agrobacterium tumefaciens), 1 against a mold (Mucor ramannianus), and 4 against a yeast (Saccharomyces pastorianus).

2. Mycotoxins in Grain

Research on mycotoxins in wheat is integrated with similar studies on corn and grain sorghum. Results are reported under Corn Utilization, Section C.

C. NEW AND IMPROVED INDUSTRIAL PRODUCTS

Research on industrial utilization of wheat starch and flour is integrated with similar work on corn starch and flour. Results are reported under Corn Utilization, Section B.

Publications

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling and Fractionation

Anderson, R. A., and Pfeifer, V. F. 1970. Stability of vitamin A in wheat flour. Northwest. Miller 277(3), pp. 14, 16-18.

Huebner, F. R. 1970. Comparative studies on glutenins from different classes of wheat. J. Agr. Food Chem. 18(2), pp. 256-259.

Krull, L. H., and Wall, J. S. 1969. Relationship of amino acid composition and wheat protein properties. Bakers Dig. 43(4), pp. 30-34, 36, 38-39.

Krull, L. H., and Wall, J. S. 1969. Chemical modification of wheat gluten with hydrazine. Can. J. Biochem. 47(6), pp. 581-585.

Mattern, P. J., Johnson, V. A., and Schmidt, J. W. (University of Nebraska, Lincoln, Nebraska). 1970. Air-classification and baking characteristics of high-protein Atlas 66X Comanche lines of hard red winter wheat. Cereal Chem. 47(3), pp. 309-316.

Rothfus, J. A. 1969. Reagent for detection of S-β-aminoethylcysteine. Anal. Biochem. 30(2), pp. 279-283.

Rothfus, J. A., and Kennel, S. J. 1970. Properties of wheat beta-amylase adsorbed on glutenin. Cereal Chem. 47(2), pp. 140-146.

Seckinger, H. L., and Wolf, M. J. 1970. Electron microscopy of endosperm protein from hard and soft wheats. Cereal Chem. 47(3), pp. 236-243.

B. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Microbiology of Cereal Flours

Hesseltine, C. W., Graves, R. R., Rogers, R., and Burmeister, H. R. 1969. Aerobic and facultative microflora of fresh and spoiled refrigerated dough products. Appl. Microbiol. 18(5), pp. 848-853.

Hesseltine, C. W., Haynes, W. C., Wickerham, L. J., and Ellis, J. J. 1969. ARS Culture Collection. Bull. Microbiol. Inst. Czech. 16(2), pp. 10-12.

Kurtzman, C. P., and Hesseltine, C. W. 1970. Chlorine tolerance of microorganisms found in wheat and flour. Cereal Chem. 47(3), pp. 244-246.

Kurtzman, C. P., Wickerham, L. J., and Hesseltine, C. W. 1970. Yeasts from wheat and flour. Mycologia 62(3), pp. 542-547.

Wickerham, L. J. 1969. Yeast taxonomy in relation to ecology, genetics, and phylogeny. Antonie van Leeuwenhoek, Yeast Symp. 1969. Suppl., Part I, 35, pp. 31-58.

2. Mycotoxins in Grains

Publications are reported under Corn Utilization, Section C.

C. NEW AND IMPROVED INDUSTRIAL PRODUCTS

Publications are reported under Corn Utilization, Section B.

GRAIN SORGHUM UTILIZATION

Problems and Objectives

The growing importance of grain sorghum as a cash crop and the increasing magnitude of production challenge technology to establish a pattern of utilization that will maintain or increase the economic value of the crop. Because the bulk of the U.S. crop is grown in a relatively limited region west of the Mississippi River, there are geographic areas where freight transportation advantages should permit increased food and industrial usage of grain sorghum without significant displacement of other cereal grains. Food usage, now taking only about 1 percent of production, could be increased by improving quality, acceptability, and suitability of grain sorghum products for food. Limited industrial outlets for grain sorghum already exist. Processing techniques specifically adapted to grain sorghum should result in improved products that can maintain present industrial outlets against competition from synthetics as well as lead to increased industrial utilization.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Get needed data on composition and processing treatments for use in making new food products from grain sorghum.

2. Convert grain sorghum to new or improved industrial products via technology suited to the specific characteristics of this grain.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Grain Sorghum Endosperm Products for Food Applications

Purpose: Although sorghum generally costs about 10 percent less than corn, only about 6 percent of the sorghum grain produced is used in U.S. food in contrast to 9 percent of the corn production. Over the next 10 years, population increases will generate increased demand for wheat-based food products in the principal grain-sorghum producing areas of the U.S. This increased demand could be supplied by supplementing wheat now required for these products with grain sorghum. To increase utilization of grain sorghum in food, compositional and genetic studies, new or improved methods of processing grain sorghum, and preparation of new or improved sorghum food products are needed.

Progress: A relatively simple process has been developed for making grits from grain sorghum using conventional equipment. Tests on several different types of grain sorghum (yellow milo hybrids, white hybrids, and yellow endosperm hybrids) have resulted in recovery of 70 to 75 percent of the grain as prime products, +14 and +20 grits, the grits having 0.5-0.8 percent fat and 0.3-0.6 percent fiber.

Conformations of prolamins from four varieties of grain sorghum were studied by optical rotatory dispersion in several solvents. In 60 percent t-butanol, the prolamins have alpha-helix contents of 40-47 percent from Moffitt parameters. There is no significant varietal difference. Addition of 1.5 M guanidine hydrochloride to 60 percent t-butanol lowers the helix content of the prolamins in general except one from a colored variety. In higher guanidine hydrochloride concentrations, the helix content is greatly reduced. Infrared spectrum of one prolamin in 60 percent t-butanol and deuterium oxide indicated the presence of alpha helix and unordered structure. The circular dichroism and far UV optical rotatory dispersion data showed characteristic alpha-helix maxima and minima.

A method to calculate automatically molecular weight from ultracentrifuge sedimentation equilibrium experiments was developed. The system consists of an ultracentrifuge equipped with a photoelectric scanner, an analog-to-digital converter, a paper-tape punch, and a system of relays. The data are processed by a computer. Several proteins of known molecular weights gave satisfactory values with this system.

Two lines of L-forms of Bacillus subtilis have been prepared under a grant to Iowa State University, Ames, Iowa. These forms were found to excrete amylase when grown on starch showing that the cell wall is not essential for amylase formation. The Pseudomonas stutzeri amylase which produces high yields of maltotetraose from starchy substrates has been isolated in crystalline form. Two active fractions were found by gel filtration. The two forms apparently have a monomer-dimer relationship. The role of zinc in the structure of B. subtilis amylase is becoming clearer. The dimer form of the amylase is chelated by zinc whereas the monomer is zinc free. The monomer is subject to neutral proteinase action, but the dimer form is not attacked. This behavior is apparently due to the fact that the zinc binding sites of the dimer are occupied, rendering it insensitive to the zinc metallo-proteinase which attacks the monomer.

In research under a grant to the University of Arkansas, Fayetteville, Arkansas, malto-oligosaccharides substituted in the 4-O-position of the terminal glucose at the nonreducing end were tested for their effect on beta-amylase action. Competitive inhibition was found with 4-O-methyl malto-oligosaccharides, and inhibition constants (K_i) for oligosaccharides of DP-2 to DP-5 have been determined. In general,

the value of K_i is inversely proportional to the degree of polymerization. 4-Deoxy malto-oligosaccharides were not attacked by beta-amylase. Further studies on Bacillus subtilis alpha-amylase showed little effect of temperature from 0° to 40° C. on the action pattern of this enzyme indicating that the free energy of binding of starch to enzyme is very small.

Grant research at Kansas State University, Manhattan, Kansas, revealed that the incorporation of hexose residues into glucoamylases I and II of Aspergillus niger involves a nucleotide hexose pathway. Hydrolysis of the heterogeneous trisaccharide units isolated from glucoamylase II with jack bean α-mannosidase showed that a substantial portion is terminated by α-D-mannoside residues.

2. Other Relevant Research

Studies on grain sorghum directed to lower cost conversion of starch to sugars and sirups and to microbial sources of rennin are integrated with similar studies on corn. Results are reported under Corn Utilization, Sections A-2 and A-4, respectively.

B. FEED, TEXTILE AND INDUSTRIAL PRODUCTS

Research in this category on grain sorghum is integrated with similar studies on corn. Results are reported under Corn Utilization, Section B.

C. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

Microbiological studies relevant to grain sorghum utilization are integrated with similar studies on corn and wheat. Results are reported under Corn Utilization, Section C, and Wheat Utilization, Section B.

Publications

A. NEW AND IMPROVED FOOD PRODUCTS

1. Grain Sorghum Endosperm Products for Food Applications

Anderson, R. A., and Wu, V. Y. 1969. Current sorghum research. Sorghum Newsletter 12, pp. 32-33.

Anderson, R. A. 1969. Producing quality sorghum flour on wheat milling equipment. Northwest. Miller 276(10), pp. 10-12, 14-15.

Anderson, R. A., Conway, H. F., Pfeifer, V. F., and Griffin, E. L., Jr. 1969. Roll and extrusion-cooking of grain sorghum grits. Cereal Sci. Today 14(11), pp. 372-375, 381.

Anderson, R. A., Jones, R. W., and Inglett, G. E. 1970. Current research on grain sorghum. Sorghum Newsletter 13, pp. 21-22.

Anderson, R. A., Montgomery, R. R., and Burbridge, L. H. 1969. Low-fat endosperm fractions from grain sorghum. Cereal Sci. Today 14(11), pp. 366-368.

Jones, R. W., and Beckwith, A. C. 1970. Proximate composition and proteins of three grain sorghum hybrids and their dry-mill fractions. J. Agr. Food Chem. 18(1), pp. 33-36.

Jones, R. W., Beckwith, A. C., Khoo, U., and Inglett, G. E. 1970. Protein composition of proso millet. J. Agr. Food Chem. 18(1), pp. 37-39.

Lineback, D. R., Russell, I. J., and Rasmussen, C. (University of Nebraska, Lincoln, Nebraska). 1969. Two forms of the glucoamylase of Aspergillus niger. Arch. Biochem. Biophys. 134(2), pp. 539-553.

Moore, C. A.,[1] and Majors, K. R.[2] ([1]USDA Economic Research Service, Peoria, Illinois; [2]USDA Federal Extension Service, Peoria, Illinois). 1970. Economic considerations. In "Sorghum Production and Utilization," eds. Joseph S. Wall and William M. Ross, chap. 18, pp. 627-664. Westport, Connecticut.

Moore, C. A.,[1] and Nelson, D. G.[2] ([1]USDA Economic Research Service, Peoria, Illinois; [2]Grain Sorghum Producers Association, Amarillo, Texas). 1970. The future of grain sorghum. In "Sorghum Production and Utilization," eds. Joseph S. Wall and William M. Ross, chap. 19, pp. 665-686. Westport, Connecticut.

Peplinski, A. J., and Pfeifer, V. F. 1970. Gelatinization of corn and sorghum grits by steam-cooking. Cereal Sci. Today 15(5), pp. 144, 149-151.

Sastry, L. V. S., and Virupaksha, T. K. (Indian Institute of Science, Bangalore, India). 1969. Alcohol-soluble proteins of grain sorghum. Cereal Chem. 46(3), pp. 284-293.*

Shoup, F. K., Deyoe, C. W., Skoch, L., Shamsuddin, M., Bathurst, J., Miller, G. D., Murphy, L. S., and Parrish, D. B. (Kansas State University, Manhattan, Kansas). 1970. Amino acid composition and nutritional value of milled fractions of sorghum grain. Cereal Chem. 47(3), pp. 266-273.

Wall, J. S., and Blessin, C. W. 1969. Composition and structure of sorghum grains. Cereal Sci. Today 14(8), pp. 264-266, 268-270, 276.

Wall, J. S., and Blessin, C. W. 1970. Composition of sorghum plant and grain. In "Sorghum Production and Utilization," eds. Joseph S. Wall and William M. Ross, chap. 4, pp. 118-166. Westport, Connecticut.

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SOYBEAN UTILIZATION

Problems and Objectives

The worldwide need for dietary protein and for food fats poses a problem that urgently demands solution. U.S. soybeans could play a dominant role in alleviating these shortages in developing countries and elsewhere around the world, if soybean protein products can be successfully used in food products tailored to meet the various nutritional and palatability requirements and if opportunities for foreign utilization of soybean oil can be increased by development of inexpensive processes for insuring flavor stability. Solution of these problems would also contribute to increased domestic use of soybean food products and soybean oil. Nonfood usage of soybean oil has rather consistently accounted for about 10 percent of domestic disappearance. In view of the increasing production of soybeans and soybean oil, maintenance of nonfood markets requires continuing effort to develop products that can compete with synthetics derived from nonagricultural sources.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Producing high-protein foods based on full-fat soybean flour.
2. Removing objectionable flavors and other factors from soy products.
3. Increasing flavor stability of soybean oil.
4. Improving refining processes for soybean oil by reducing cost and associated water pollution.
5. Converting soybean oil to competitive industrial chemical products.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Development of Processes for Improving Flavor and Acceptability of Edible Soybean Products

   Purpose: Despite their content of high-quality protein, soybean products have found only limited outlets in foods in the United States. Flavor of soy products frequently is unacceptable to consumers. The tendency to produce flatulence in some persons and the possible

presence of trypsin inhibitor or other antinutritional factors are also significant disadvantages. Because of the potential importance of soybean products in improving the adequacy of diets of many segments of the U.S. population, processes are urgently needed for producing products that have acceptable flavor and color, that contain no undesirable antinutritional factors, and that retain the functional properties that are critical to use of these products in many application.

Progress: Commercial soy flours, concentrates, and isolates were evaluated for odor and flavor by a 17-member taste panel. Odor responses included beany, cereal, musty, and toasted; and flavor descriptions were beany, bitter, cereal, and astringent. Flavor thresholds on selected samples showed a reduction in flavor in isolates and concentrates as compared to a flour. Hexane-defatted soybean meals were reextracted with hexane-alcohol azeotropic mixtures to obtain products with higher flavor scores. Isolates from azeotrope-extracted meals also had higher flavor scores than did isolates from hexane-extracted meals.

Ethyl-alpha-D-galactopyranoside and pinitol have been isolated from dehulled, full-fat soybean flakes by extraction with an azeotropic mixture of hexane-ethyl alcohol (79:31). The azeotrope solvent is effective in extracting most of the flavor in full-fat and defatted flakes. The two compounds were identified by carbon-hydrogen analysis, chromatographic behavior, spectroscopy, and other tests. Ethyl-alpha D-galactoside has been synthesized and is identical to that isolated from soybeans. Ethyl galactoside has a bitter taste and pinitol is somewhat sweet. On the basis of volatile carbonyl compound analysis and thiobarbituric acid assay, it was found that very little lipid oxidation occurs during the processing of soybeans into soy flour and protein. Since no rancid odors or flavors developed during the preparation of these products, the green beany, bitter flavors, characteristic of soy products, may pre-exist in the mature seed. Taste panel evaluation of developing soybeans has shown that the green beany, bitter flavors are also present in the very immature seeds.

In work supported by NIH under the U.S.-Japan Cooperative Medical Program, the most promising strains of Streptomyces reported by Pridham and Lyons for production of alpha-galactosidase were tested for their ability to produce the enzyme on a soybean-based nutrient medium. About 10 cultures produced appreciable amounts of alpha-galactosidase. None of the Streptomyces investigated produced enough alpha-galactosidase to be of interest commercially. Representative strains of several Aspergillus species were then tested. Most all of these strains showed ability to hydrolyze raffinose but only about 25 percent

showed action on melibiose. A commercial product derived from an Aspergillus species is now offered for sale and is said to contain alpha-galactosidase. Tests of the product on raffinose and soybean meal show that it releases monosaccharides according to the claim made by the manufacturer.

The production of conjugated hydroxyoctadecadienoic acids from vegetable oil soap stocks by lipoxygenase oxidation is a promising development resulting from research on characterization of soybean lipoxidases. The substrate concentration used in the standard lipoxygenase assay method has been increased 500 times to allow 100 mg. per ml. (10% solution) of soap stock to be used. The procedure produces 70 to 90 percent oxidation in 20 to 40 minutes and can be used with either distilled or crude soap stock. After hydroperoxide reduction, the oxidized soybean soaps contained 40 to 45 percent of conjugated hydroxy dienes. Defatted soy flour or crude aqueous extracts of soy flour are inexpensive and effective enzyme sources.

In grant studies at Baylor University College of Medicine, Houston, Texas, mature dogs were maintained on a 15 percent raw soybean diet for up to 16 weeks prior to sacrifice. Little change in body weight occurred. There was only a slight increase in pancreas weight, and protein content of the pancreas remained nearly constant. Most of the enzymes showed a similar response pattern, i.e., decreasing in activity at the 4-6 week period. At the end of the feeding tests, the concentrations of trypsin, chymotrypsin, and lipase in the pancreas were only slightly elevated compared with the control dogs. Amylase activity remained fairly constant throughout. Younger dogs with permanent pancreatic cannulae showed an increased pancreatic juice secretion when fed 15 percent raw soybean meal diet.

Grant research at the University of Illinois showed that dogs fed an all-meat diet produced very little gas compared to those fed either dog food containing soy grits or a basal diet supplemented with defatted soy flour. The increase in flatus volume for the soybean diets was more than 3-fold. The microflora changed markedly in the 15-day feeding trial in dogs fed soybeans. When the all-meat and soybean diets were reversed in a following 15-day feeding trial, changes in flatus volume and microflora were also reversed. Fecal analysis of dogs fed a flatulent soybean diet showed that the anaerobic bacteria associated with the production of CO_2 and H_2 increased greatly and accounted for the increase in flatulence. Mucosal samples taken from an ileo- and colonic-fistula dog fed soybeans produced large volumes of gas compared with an all-meat diet.

Under a grant to the University of Minnesota, 26 soybean varieties were analyzed for trypsin inhibitor, chymotrypsin inhibitor, and hemagglutinating activities plus methionine and cystine contents. Raw meals

from the 26 varieties were fed to rats to determine protein efficiency ratios (PER) and effects on pancreas weights. Pancreas weights correlated inversely with PER; the other parameters showed no correlation with PER.

Research directed towards improvement of the flavor and acceptability of soybean food products is also conducted under PL 480 grants to several foreign institutions. At the University of Tokyo, Tokyo, Japan, ground raw soybeans and defatted soy flour were analyzed for volatile constituents and phenolic acids. Numerous compounds that were found are as follows: 17 carbonyl compounds, 9 carboxylic acids, 9 phenolic acids, 3 amines, 1 ester, 7 alcohols, ammonia and hydrogen sulfide. n-Hexanol and n-hexanal appear to contribute to the green bean flavor of soybeans. This alcohol and aldehyde bind to the protein and are difficult to remove. Molsin, an enzyme containing aspergello-peptidase and Aspergillus carboxypeptidase, substantially reduces any bitterness and removes the original green flavor. Further work is needed to determine if these findings have any practical significance in commercial operations. This research is now completed.

Another completed study at the University of Tokyo showed that the 7S fraction of soybean protein gives a much softer tofu than does the 11S. Since varieties of soybeans vary from 0.56 to 1.3 in the ratio of 7S to 11S, the variety may make a definite difference in tofu manufacture. Phytic acid content also affects hardness of tofu. Dried tofu browns at an accelerated rate when iron and copper are present. Particle size of coagulant used in making the fresh tofu influences texture of dried tofu.

Scientists at the Weizmann Institute of Science, Rehovoth, Israel, have improved isolation and purification of the glycopeptide from soybean hemagglutinin by digesting hemagglutinin with highly purified pronase at 50° C. and chromatographing the hydrolysate on Dowex 50-X2. The ratio of aspartic acid:mannose:N-acetylglucosamine in the resulting glycopeptide was 1:9:2-3. Molecular weight of the glycopeptide was approximately 2000. An additional glyco protein different from hemagglutinin has been isolated from soybean whey.

Lipase production by strains of Pseudomonas has been studied at the University of Baroda, Baroda, India, and the nutritional requirements are now known. Glutamic acid, proline, tyrosine, alanine, leucine, lysine, and valine support both growth and lipase production. Addition of some triglycerides and fatty acids to the medium increased growth but did not increase lipase production. Apparently the lipase of these organisms is not induced by substrate. The lipase can be purified by precipitation with 50 percent ammonium sulfate. It is quite thermolabile, losing 85 percent of its activity upon heating

at 60° C. for 10 minutes. The properties and mode of action of this lipase do not distinguish it from previously described lipases from Pseudomonads.

In research completed during the year at Kagawa University, Takamatsu Japan, it has been concluded that soybeans contain about 5 percent sucrose, 1 percent raffinose, and 4 percent stachyose. Sucrose is digestible, but human digestive enzymes are unable to hydrolyze the alpha-galactoside linkage of raffinose and stachyose. Screening studies on intestinal bacteria showed that certain strains of Escherichia coli and Streptococcus faecalis have appreciable alpha-galactosidase activity. The alpha-galactosidase of E. coli was isolated and characterized. The purified enzyme had a pH optimum of 6.8 and optimum temperature for activity was 37° C. Rats fed soybean oliogosaccharides absorbed about 90 percent of the sugars but absorption dropped to 52 percent when the diet included antibiotics to suppress intestinal bacteria. Significant amounts of raffinose and stachyose from soybeans may therefore be digested by humans as a result of bacterial enzyme action in the digestive tract.

2. Selective Hydrogenation of Soybean Oil

Purpose: Flavor instability of soybean oil has been attributed to the presence of linolenic acid among the component fatty acids of the oil. Linolenate can be removed by selective hydrogenation. However. when conventional nickel hydrogenation catalysts are used, excessive amounts of saturates and undesired trans isomers are formed. Research at the Northern Division is concerned with finding catalysts that will selectively remove linolenate while preserving the maximum polyunsaturation in the other fatty acids and minimizing the formation of saturates and trans isomers. Certain catalysts containing copper have been found to display a high degree of selectivity and are currently being intensively studied.

Progress: Investigations were conducted on the organoleptic and stability evaluation and on the trace metal analysis of different lots of soybean oil hydrogenated with copper catalysts under various conditions. Taste panel results show that excellent flavor and oxidative stabilities are obtained as the linolenate content is decreased to low levels. Painty and fishy responses decrease directly as the linolenate content is lowered. At zero linolenate, fishy and painty responses are very low, and the flavor responses are typical of accepted cooking oils. Copper-reduced soybean and cottonseed oils were scored significantly better than a nickel-reduced soybean oil (3% linolenate) after the 200° C. heat test. The apparent absence of "hydrogenation" flavor in copper-reduced oils is a further advantage of these products. Residual copper remaining in the oil after hydrogenation is easily

removed to innocuous levels (less than 0.02 p.p.m.) by bleaching. Unsaturated aliphatic hydrocarbons, previously ignored as a source of oxidized and off-flavors in autoxidized fats, were found to be detectable by odor and taste at very low concentrations (less than 1 p.p.m.) in cottonseed oil.

Four plant-scale tests of the partial hydrogenation of soybean oil with a commercially produced copper-chromite catalyst were conducted with the cooperation of an industrial producer of soybean shortening and salad oil. The objective was to selectively reduce linolenate but not to reduce linoleate. The Northern Division provided information for selecting an active catalyst, pretested the catalyst for activity, and developed operating procedures for the tests. The linolenate-linoleate selectivity attained in plant tests was lower than that attained in pilot-scale tests with the same catalyst, but it was substantially higher than the selectivity attained with a commercial nickel catalyst under the same conditions of temperature and hydrogen pressure. The lower selectivity was apparently caused by contamination of the reactions with small amounts of nickel catalyst from other plant operations. The product oil, after winterization and deodorization, was of very good edible quality.

A microreactor has been built to collect odor materials from the air over soybean oil at frying temperatures. The odor material is injected directly onto a frozen GLC column where it does not move until the column is temperature programmed, thus giving good GLC resolution. The GLC column has three outlets--a flame ionization detector, the mass spectrometer, and a vent to the room to allow a scientist to sniff the odors.

Additional information on the mechanism of hydrogenation with copper catalysts obtained by reduction of conjugated esters with deuterium show that reduction occurs by 1,2, 1,4, and 1,6 addition. A simple method has been devised to prepare methyl trans-10,cis-12 octadecadienoate for use as a model compound.

In contract research at the University of Illinois, Urbana, Illinois, organometallic complexes found active at hydrogenation catalysts include $/Et_4N/$ $/Mo_2(CO)_4(C_3H_5)_2Cl_3/$, $Mo(Ph_3As)_2(CO)_3Cl_2 + SnCl_2$, $/C_3H_5PdCl/2$ and $PdCl(\pi\text{-}C_3H_5)PPh_3$. The molybdenum compounds were selective for hydrogenation of polyunsaturates in soybean esters but the palladium compounds were not. Insoluble bimetallic polyphthalocyanines of Pd and Cu and of Pt and Fe were only weakly active as heterogeneous hydrogenation catalysts.

In research at the Sugiyama Chemical Research Institute, Tokyo, Japan, tocored, the oxidation product of gamma tocopherol is suspected of being the main cause of color reversion in soybean oil. A maximum amount of tocored occurs in crude oil extracted from high moisture beans (15-18%). Most of the tocored is removed from the oil during refining, but about 30 percent remains in a colorless form in the oil. This colorless form undergoes oxidation by air upon storage to form tocored and the brown color of reverted oil. Tocored is also formed by oxidation of the tocopherol dimers. Subsequent oxidation of tocored produces a variety of red and purple compounds. Color formation is coupled to the autoxidation of the oil. This research, completed during the year, was conducted under a PL 480 grant.

Under a PL 480 grant to the University of Bombay, Bombay, India, a number of pure synthetic triglycerides containing palmitic, stearic, elaidic, oleic, and linoleic acid were prepared by acetylation of 1,3-diglycerides (palmitic, elaidic, and stearic acids). These compounds will serve as standards for use in identification of components of hydrogenated soybean oil.

At the Hebrew University of Jerusalem, Jerusalem, Israel, work has continued on the isolation, purification, and characterization of sterols and triterpenes in soybean oil. Eight triterpenes have been isolated by chromatographic techniques and 5 of them identified. This research is being conducted under a PL 480 grant.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Lubricants

Purpose: The problem of providing adequate lubrication for machines under new and extreme conditions is assuming even greater importance. The trend is toward permanent lubrication or longer periods between relubrications of machinery. Hydrocarbon oils from petroleum are unable to do the job alone; hence a large number of additives have been utilized. Alternatively, various synthetic lubricants have been developed. Among lubricants other than hydrocarbons, compounds with longer chains attached to or between polar functional groups are generally more effective, other factors being equal, than those with shorter chains. Possibilities exist to develop from vegetable oils

lubricants that can meet modern technological needs at less cost than presently used synthetics or hydrocarbon oils plus additives. These possibilities are being systematically explored and evaluated.

Progress: Excellent yields of ester dialdehyde or diester acid have been obtained from the propylene adduct of isomeric methyl linoleate by ozonization followed by reduction or thermal decomposition. The main byproduct formed from the decomposition at 25° C. is an isomeric methyl carboalkoxystearate. Decomposition at higher temperatures (180° C.) also yields the methyl ester of methylstearic acid. Viscosity data at 100 and 210° F. obtained on the triester, 2,2-dimethylpentyl dicarbomethoxystearate, meet the lubrication specifications of MIL-L-23699. The physical properties of partially hydrogenated soybean and linseed oils, and trimethylolpropane, trimethylolethane, and pentaerythritol esters of partially hydrogenated soybean fatty acids have been determined. These compounds are being studied to evaluate their potential for processing of steel.

2. Nylon-Type Polyamides

Purpose: Nylon-9 has the highest melting point of nylons characterized by low moisture absorption. Of this group of nylons, nylon-11 and-12 are manufactured abroad and imported into the U.S. If available domestically at a competitive price, nylon-9 should replace these imported nylons and find ready market acceptance. Work at the Northern Division has shown that soybean oil is a preferred starting point for a synthetic route to nylon-9 that should be technically and economically feasible. The synthesis involves alcoholysis of the oil to alkyl soyate which is ozonized to a compound that is converted in one step to alkyl 9-aminononanoate. The Division's current program comprises process and product development of nylon-9.

Progress: Steps in the process for preparing nylon-9 from soybean oil were standardized to assure reliability and reproducibility in further developmental work now being carried out. A major problem is purification of 9-aminononanoic acid to form a product suitable for polymerization. A possible alternate route to the amino acid would make use of soybean fatty acids recovered from foots formed during alkali refining of the oil. Preliminary experiments indicated an overall conversion to crude amino acid of about 48 percent. Further study is needed to evaluate the potential of this route. In studies with byproducts to improve the overall economics of the nylon-9 synthesis, hexanal was converted in about 40 percent yield to the potentially useful intermediate, 2,2-bis(hydroxymethyl)hexanal by condensation with formaldehyde.

In basic research directed towards possible development of other types of polyamide resins, aldehyde esters were obtained in 80 to 99 percent yields by hydroformylation of methyl oleate with both soluble and supported rhodium catalysts in the presence of triphenylphosphine. Methyl linoleate was 40 to 60 percent conjugated with molybdenum and tungsten carbonyls.

Contract research at Southern Research Institute, Birmingham, Alabama, has essentially confirmed the results obtained by the Northern Division in carrying out the synthesis of nylon-9 from soybean oil, and has scaled the synthesis up to pound-size batches. Preparation of amino acid in pure form is recognized as the major problem. Improvements have been effected in amount of palladium catalyst required for hydrogenation of the ozonolysis products, reuse of catalyst, and simplification of procedures in general. Encouraging results have been obtained from preliminary investigations of an alternate route that starts with soy nitriles. The 9-aminoononanoic acid from this route may be somewhat more easily purified than that from the route originally developed at the Northern Division.

C. FOREIGN MARKET DEVELOPMENT

1. High-Protein Foods from Full-Fat Soybean Flours

Purpose: This research was originally undertaken as part of the Food for Peace Program under UNICEF and AID sponsorship to help alleviate the dietary deficiency of protein in developing countries. Work is now redirected towards the developing of cash markets in countries that are becoming increasingly able to pay dollars for U.S. soybeans. A new process is being developed for the manufacture of full-fat soybean flour based on high-temperature, short-time extrusion cooking. Solutions of problems involved in utilization of the full-fat flour, e.g. in beverages, are also being sought.

Progress: Further studies on the utilization of full-fat soy flour in beverage products have resulted in development of a soybean-base beverage powder that has good shelf life and that disperses in water to a nutritious, creamy, pale-yellow liquid with good flavor and beverage characteristics. The dry formulation made by a special wet-milling and spray-drying process contains 33 percent protein, 30 percent fat, and 28 percent carbohydrate. It is based on a blend of extrusion-cooked full-fat soy flour, hydrogenated soybean oil, emulsifier, and cream flavoring, plus vitamins and minerals. A preliminary cost study shows that one pound of the dry beverage base, which reconstitutes to one gallon of beverage, can be produced for less than 10 cents per pound, exclusive of marketing costs and profit.

The new beverage products represents a substantial advance over previous types of soybean-based beverages. In particular, the graininess associated with such beverages has been virtually eliminated, and dispersibility and the stability of the suspension to settling have been greatly improved. The product has been very well accepted in tests conducted by the UTTA-Pradish Agricultural University group at Pant Nagar, India. This group would like to use the process in their high-protein food program. One U.S. industrial company has expressed interest in the product for India in connection with its AID contract.

D. ALLEVIATION OF WATER POLLUTION

1. Soybean Oil Refining Process

Purpose: In alkali-refining edible soybean oil, the crude oil is mixed with an aqueous solution of caustic soda and the aqueous phase containing phosphatides and fatty acid soaps is separated. The refined oil is then washed with water to further remove soap. This wash water which also contains emulsified oil is sent to municipal sewage disposal plants or discarded into rivers and streams where it contributes to water pollution. The refined and washed oil is dried and then heated with clay at an elevated temperature. The principal reason for this step, although it is termed "bleaching," is to further remove traces of soap which, if not removed, give a poor taste to the oil and also poison hydrogenation catalysts. The Northern Division has devised a process for washing alkali refined oil in which water is passed through the oil, then through ion-exchange resin and recycled. This process eliminates the problem of disposing of alkaline, soap-containing wash water, avoids loss of oil due to emulsions, and removes soap so effectively that the bleaching step can be omitted. Current research is directed towards developing the process to a point such that trials can be made in an operating soybean oil refining plant.

Progress: Water-recycle washing of alkali-refined, unwashed soybean oil was further investigated in a vertical rotating disc-countercurrent contactor, and in a cocurrent mixer and centrifuge. About equally satisfactory alkali reduction was achieved with both types of equipment. The cocurrent mixer-centrifuge is the type of system employed in commercial refineries for washing alkali-refined vegetable oils. Therefore, with only the addition of ion-exchange equipment and accessories, washing equipment now in use can be converted to water-recycle operation with expectation of satisfactory results in terms of adequate sodium removal and complete recycle and reuse of wash water.

Publications and Patents

A. NEW AND IMPROVED FOOD PRODUCTS

1. Development of Processes for Improving Flavor and Acceptability of Edible Soybean Products

Arai, S., Suzuki, H., Fujimaki, M., and Sakurai, Y. (University of Tokyo, Tokyo, Japan). 1966. Studies on flavor components in soybean. Part III. Volatile fatty acids and volatile amines. Agr. Biol. Chem. (Tokyo) 30(9), pp. 863-868.*

Cowan, J. C. 1969. Soybeans. In "Kirk-Othmer Encyclopedia of Chemical Technology," 2nd ed., vol. 18, pp. 599-614.

Dimler, R. J. 1969. Oilseed proteins. Chem. Eng. Progr. 65(9), pp. 20-26.

Eldridge, A. C. 1969. A bibliography on the solvent extraction of soybean products 1944-1968. J. Amer. Oil Chem. Soc. 46(9), pp. 458A, 460A, 462A, 464A, 496A, 498A, 500A, 502A.

Eldridge, A. C., Kalbrener, J. E., and Wolf, W. J. 1970. Soybean trypsin inhibitor: A reference protein for gel electrophoretic studies of soybean proteins. Cereal Chem. 47(2), pp. 167-172.

Eldridge, A. C., and Wolf, W. J. 1969. Crystalline saponins from soybean protein. Cereal Chem. 46(4), pp. 344-349.

Eldridge, A. C., and Wolf, W. J. 1969. Polyacrylamide-gel electrophoresis of reduced and alkylated soybean trypsin inhibitors. Cereal Chem. 46(5), 470-478.

Honig, D. H., Sessa, D. J., Hoffmann, R. L., and Rackis, J. J. 1969. Lipids of defatted soybean flakes: Extraction and characterization. Food Technol. 23(6), pp. 95-100.

Ishaaya, I., Birk, Y., Bondi, A., and Tencer, Y. (The Hebrew University of Jerusalem, Rehovoth, Israel). 1969. Soybean saponins. IX. Studies of their effect on birds, mammals and cold-blooded organisms. J. Sci. Food Agr. 20(7), pp. 433-436.*

Kawamura, S., and Kasai, T. (Kagawa University, Takamatsu, Japan). 1968. Decomposition of soybean oligosaccharides by intestional bacteria. II. Comparison of twenty strains of Escherichia coli for consuming each sugar in the sugar mixture extracted from defatted soybean meal. Tech. Bull. Fac. Agr., Kagawa Univ. 20(1), pp. 33-40.*

*Research supported by PL 480 funds.

Kawamura, S., and Kasai, T. (Kagawa University, Takamatsu, Japan). 1968. Decomposition of soybean oligosaccharides by intestinal bacteria. III. Comparison of eighteen strains of Escherichia coli for consuming some mono- and oligosaccharides. Tech. Bull. Fac. Agr., Kagawa Univ. 20(1), pp. 41-48.*

Kawamura, S., Miyake, T., and Narasaki, T. (Kagawa University, Takamatsu, Japan). 1968. Decomposition of soybean oligosaccharides by intestinal bacteria. I. General introduction and preliminary screening of some strains of Escherichia coli for the ability of decomposing raffinose. Tech. Bull. Fac. Agr., Kagawa Univ. 20(1), pp. 25-32.*

Lis, H., Sharon, N., and Katchalski, E. (Weizmann Institute of Science, Rehovoth, Israel). 1969. Identification of the carbohydrate-protein linking group in soybean hemagglutinin. Biochim. Biophys. Acta 192(2), pp. 364-366.*

Lyons, A. J., Jr., Pridham, T. G., and Hesseltine, C. W. 1969. Survey of some Actinomycetales for α-galactosidase activity. Appl. Microbiol. 18(4), pp. 579-583.

Mori, H., and Onishi, H. (Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan). 1969. Breeding and stability of diploid Saccharomyces rouxii. J. Ferment. Technol. 47(6), pp. 394-397.*

Sessa, D. J., Honig, D. H., and Rackis, J. J. 1969. Lipid oxidation in full-fat and defatted soybean flakes as related to soybean flavor. Cereal Chem. 46(6), pp. 675-686.

Teeter, H. M., and Schaefer, W. C. 1969. Food uses of soybeans. Research at the Northern Laboratory. Soybean Dig. 30(1), pp. 16-18.

Thomas, P. J., and Dutton, H. J. 1969. Improved resolution in high-sensitivity dual-label gas radiochromatography. Anal. Chem. 41(4), pp. 657-660.

Thomas, P. J., and Dutton, H. J. 1970. Preparation and counting of lipophilic samples. In "The Current Status of Liquid Scintillation Counting," ed. Edwin D. Bransome, Jr., pp. 164-169. New York.

Wang, L. C., Smith, A. K., and Cowan, J. C. 1969. A note on the detection of leucoanthocyanins in defatted soybean flakes. Cereal Chem. 46(5), pp. 468-470.

Wang, L. C. 1969. Effect of alcohol washing and autoclaving on nucleotides of soybean meal. J. Agr. Food Chem. 17(2), pp. 335-340.

*Research supported by PL 480 funds.

Wang, L. C., and Anderson, R. L. 1969. Purification and properties of soybean allantoinase. Cereal Chem. 46(6), pp. 656-663.

Wolf, W. J. 1969. Chemical and physical properties of soybean proteins. Bakers. Dig. 43(5), pp. 30-31, 34-37.

Wolf, W. J. 1970. Scanning electron microscopy of soybean protein bodies. J. Amer. Oil Chem. Soc. 47(3), pp. 107-108.

Wolf, W. J., Rackis, J. J., and Smith, A. K. 1969. Chemistry of soybean proteins. In "Chemical and Physical Aspects of Food," ed. James Muil Leitch, Vol. I, Proc. First Int. Congr. Food Sci. Technol., London, 1962, pp. 131-141.

Wolf, W. J., and Tamura, T. 1969. Heat denaturation of soybean 11S protein. Cereal Chem. 46(4), pp. 331-344.

Wolf, W. J., and Thomas, B. W. 1970. Thin layer and anion exchange chromatography. J. Amer. Oil Chem. Soc. 47(3), pp. 86-90.

2. Selective Hydrogenation of Soybean Oil

Beal, R. E., Moulton, K. J., Moser, H. A., and Black, L. T. 1969. Removal of copper from hydrogenated soybean oil. J. Amer. Oil Chem. Soc. 46(9), pp. 498-500.

Black, L. T., and Beal, R. E. 1969. Determination of the $\Delta 15$ double bond in fatty acids of hydrogenated soybean oil. J. Amer. Oil Chem. Soc. 46(6), pp. 312-314.

Butterfield, R. O. 1969. Countercurrent and counter double current distribution of 2-methoxyethyl and 2-chloroethyl fatty esters. J. Amer. Oil Chem. Soc. 46(6), pp. 320-322.

Butterfield, R. O. 1969. Kinetic rate constants determined by a digital computer. J. Amer. Oil Chem. Soc. 46(9), pp. 429-431.

Dutton, H. J. 1970. Computer-guided explorations in lipid chemistry. J. Amer. Oil Chem. Soc. 47(3), pp. 98A, 100A, 102A, 120A, 122A.

Evans, C. D., List, G. R., Hoffmann, R. L., and Moser, H. A. 1969. Edible oil quality as measured by thermal release of pentane. J. Amer. Oil Chem. Soc. 46(9), pp. 501-504.

Evans, C. D., McConnell, D. G., List, G. R., and Scholfield, C. R. 1969. Structure of unsaturated vegetable oil glycerides: Direct calculation from fatty acid composition. J. Amer. Oil Chem. Soc. 46(8), pp. 421-424.

Fenselau, C.,[1] Young, J. L.,[1] Meyerson, S.,[2] Landis, W. R.,[3] Selke, E., and Leitch, L. C.[4] ([1]John Hopkins University, Baltimore, Maryland; [2]American Oil Company, Whiting, Indiana; [3]National Institutes of Health, Bethesda, Maryland; [4]National Research Council, Ottawa, Ontario, Canada). 1969. A cyclobutanol intermediate in the decomposition of aldehydes under electron impact. A further photochemical analogy. J. Amer. Chem. Soc. 91(24), pp. 6847-6848.

Frankel, E. N. 1970. Conversion of polyunsaturates in vegetable oils to cis-monounsaturates by homogeneous hydrogenation catalyzed with chromium carbonyls. J. Amer. Oil Chem. Soc. 47(1), pp. 11-14.

Frankel, E. N. 1970. Homogeneous catalytic conjugation of polyunsaturated fats by chromium carbonyls. J. Amer. Oil Chem. Soc. 47(2), pp. 33-36.

Frankel, E. N., and Butterfield, R. O. 1969. Homogeneous hydrogenation of diolefins catalyzed by tricarbonyl chromium complexes. I. Stereoselective 1,4 addition of hydrogen. J. Org. Chem. 34(12), pp. 3930-3936.

Frankel, E. N., and Dutton, H. J. 1970. In "Topics in Lipid Chemistry," ed. F. D. Gunstone, vol. 1, pp. 161-276. London.

Frankel, E. N., and Little, F. L. 1969. Homogeneous catalytic hydrogenation of unsaturated fats: Group VIB metal carbonyl complexes. J. Amer. Oil Chem. Soc. 46(5), pp. 256-261.

Frankel, E. N., Selke, E., and Glass, C. A. 1969. Homogeneous hydrogenation of diolefins catalyzed by tricarbonyl chromium complexes. II. Deuteration. J. Org. Chem. 34(12), pp. 3936-3942.

Koritala, S. 1970. Selective hydrogenation of soybean oil: V. A novel copper catalyst with excellent re-use properties. J. Amer. Oil Chem. Soc. 47(3), pp. 106-107.

Koritala, S. June 2, 1970. Selective hydrogenation of soybean oil with supported copper catalysts. U.S. Patent 3,515,678.

Koritala, S., and Dutton, H. J. 1969. Selective hydrogenation of soybean oil: IV. Fatty acid isomers formed with copper catalysts. J. Amer. Oil Chem. Soc. 46(5), pp. 245-248.

Moulton, K. J., Moore, D. J., and Beal, R. E. 1969. Pilot-plant selective hydrogenation of soybean oil: Activation and evaluation of copper-containing catalysts. J. Amer. Oil Chem. Soc. 46(12), pp. 662-666.

Mounts, T. L., Butterfield, R. O., Scholfield, C. R., and Dutton, H. J. 1970. Micro vapor-phase hydrogenation monitored with tandem chromatography-radioactivity: III. Isomeric monoenes. J. Amer. Oil Chem. Soc. 47(3), pp. 79-82.

Mounts, T. L., Evans, C. D., Dutton, H. J., and Cowan, J. C. 1969. Some radiochemical experiments on minor constituents in soybean oil. J. Amer. Oil Chem. Soc. 46(9), pp. 482-484.

Mounts, T. L., McWeeny, D. J., Evans, C. D., and Dutton, H. J. 1970. Decomposition of linoleate hydroperoxides: Precursors of oxidative dimers. Chem. Phys. Lipids 4(2), pp. 197-202.

Rakoff, H. 1969. Synthesis of unsaturated aldehydes. J. Amer. Oil Chem. Soc. 46(6), pp. 277-279.

Scholfield, C. R., Butterfield, R. O., Mounts, T. L., and Dutton, H. J. 1969. Relative reduction rates of fatty acid isomers by hydrazine. J. Amer. Oil Chem. Soc. 46(6), pp. 323-326.

Scholfield, C. R., and Dutton, H. J. 1970. Gas chromatographic equivalent chain lengths of isomeric methyl octadecenoates and octadecynoates. J. Amer. Oil Chem. Soc. 47(1), pp. 1-2.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Lubricants

Bell, E. W., and Cowan, J. C. May 19, 1970. Cyclic C_{18} and C_{20} alcohols. U.S. Patent 3,513,205.

Dufek, E. J., Cowan, J. C., and Friedrich, J. P. 1970. Ethylene adduct of conjugated octadecadienoic acids: II. Ozonization products. J. Amer. Oil Chem. Soc. 47(2), pp. 51-55.

Dufek, E. J., Gast, L. E., and Friedrich, J. P. 1970. Ethylene adduct of conjugated octadecadienoic acids: I. Peracid oxidation products. J. Amer. Oil Chem. Soc. 47(2), pp. 47-50.

2. Nylon-Type Polyamides

Beal, R. E. Mar. 31, 1970. Ozonization of vegetable oils in an improved aqueous medium. U.S. Patent 3,504,038.

Davison, V. L., Pryde, E. H., and Moore, D. J. Dec. 23, 1969. Thermally stable stationary phases for temperature programmed gas chromatography. U.S. Patent 3,485,028.

Kohlhase, W. L., Pryde, E. H., and Cowan, J. C. 1970. 9-Aminononanamide and nylon-9 from azelaaldehydic derivatives of soybean oil. J. Amer. Oil Chem. Soc. 47(5), pp. 183-188.

Lenz, R. W. (University of Massachusetts, Amherst, Massachusetts). 1969. Structure, properties, and cross-linking reactions of poly(ester acetals). Macromolecules 2(2), pp. 129-136.

Lenz, R. W.,[1] Awl, R. A., Miller, W. R., and Pryde, E. H. ([1]University of Massachusetts, Amherst, Massachusetts). 1970. Poly(ester-acetals) from geometric isomers of methyl azelaaldehydate glycerol acetal. J. Polym. Sci., Part A-1, 8(2), pp. 429-444.

Miller, W. R., Awl, R. A., Pryde, E. H., and Cowan, J. C. 1970. Poly(ester-acetals) from azelaaldehydic acid-glycerol compounds. J. Polym. Sci., Part A-1, 8(2), pp. 415-427.

Pryde, E. H., and Cowan, J. C. 1969. Reactions of azelaaldehydic esters. J. Amer. Oil Chem. Soc. 46(4), pp. 213-218.

C. FOREIGN MARKET DEVELOPMENT

1. High-Protein Foods from Full-Fat Soybean Flours

Mustakas, G. C., Albrecht, W. J., McGhee, J. E., Black, L. T., Bookwalter, G. N., and Griffin, E. L., Jr. 1969. Lipoxidase deactivation to improve stability, odor, and flavor of full-fat soy flours. J. Amer. Oil Chem. Soc. 46(11), pp. 623-626.

D. ALLEVIATION OF WATER POLLUTION

1. Soybean Oil Refining Process

Eisenhauer, R. A., Beal, R. E., and Griffin, E. L., Jr. 1970. Water recycle method for washing alkali-refined soybean oil. J. Amer. Oil Chem. Soc. 47(4), pp. 137-140.

FLAX UTILIZATION

Problems and Objectives

Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Recently, annual domestic use of linseed oil has declined to 325 million pounds from a postwar high of over 700 million pounds in 1950. This decrease came primarily by displacement by synthetic materials capable of better performance, particularly in coatings. To restore the level of use of linseed oil, new or expanded markets are urgently needed. The most promising route to achievement of this goal is development of improved protective coating products that can compete with synthetics. Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry.

Major objectives of current research are to develop and evaluate alternative ways for:

1. Improving durability of linseed emulsion paints.

2. Establishing economically feasible methods of curing and/or protecting concrete by use of linseed oil.

3. Converting linseed oil by economical processes to products that can compete in the industrial chemical market.

Progress Report

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Curing and Protecting Concrete

Purpose: Research at the Northern Division has demonstrated that coating concrete with boiled linseed oil applied as a solution in mineral spirits effectively protected air-entrained concrete from freeze-thaw damage and the effects of deicing chemicals. This treatment is now used by 36 States and 18 toll authorities. Later a linseed oil emulsion was devised that could be used not only to protect concrete but also to cure freshly poured concrete. The emulsion is now used commercially for this dual purpose in three States. The most recent development is that preliminary results of exploratory tests suggest that use of the emulsion to coat porous and alkali-reactive aggregates improves their performance as a

component of Portland cement concrete. Present studies are directed towards learning more about how linseed oil functions in curing and protecting concrete; overcoming various problems encountered in use of linseed oil emulsion to cure and protect concrete; obtaining additional performance data that will encourage additional States to adopt the emulsion; and exploring the value of linseed oil in upgrading unsound aggregate.

Progress: Studies of the penetration of concrete by linseed oil emulsions and solutions were continued. Concrete blocks cured with polyethylene and commercial resin curing agents were treated with linseed oil solutions containing 10 to 50 volume percent oil. Penetration patterns were similar with both cures at temperatures from 34 to 100° F. and deepest penetration resulted from the dilute solutions. "Dilute" emulsions of linseed oil give penetration similar to the 50 percent emulsion. Boiled linseed oil emulsions have been found to pass the ASTM water-loss tests when applied at 175 sq. ft./gal. and appear to show promise both as a curing and antispalling agent. Mixed emulsion (50% oil) containing M-37 and boiled linseed oils may also serve in this dual role.

In contract research at the Kansas State University, Manhattan, Kansas, five poor or marginal Kansas aggregates have been obtained and preliminary tests to measure their physical properties have been completed. Marginal aggregates from other geographical areas are being obtained and characterized. A literature survey revealed that no work has been done on use of coatings to upgrade aggregate for Portland cement concrete.

2. Improved Durability of Linseed Oil Emulsion Paints

Purpose: In recent years, the market for linseed oil in oil-based paints has greatly declined due to the popularity of synthetic latex paints for exterior use. Linseed emulsion paints were developed in response to this competition and were commercially available for several years. These paints were, however, not a commercial success because some of their properties, especially tint retention and durability during the first year, were inferior to those of the synthetic-latex paints. Lack of water permeability and flexibility are also important factors that reduce durability by leading to blistering and cracking. Research is needed to improve the properties of linseed emulsion paints and thereby enhance their competitive position. Opportunities also exist to capture a share of the market for water-based industrial coatings by developing new rapid-drying, hard, durable paint vehicles based on linseed oil.

Progress: Scanning electron microscopy of linseed oil emulsion and solvent-base coatings and of latex paints has now indicated that linseed emulsion paints are comparable to the other coatings in several respects affecting quality of films formed. The morphology of top surfaces and of the interior shows good flow and bonding between pigment and binder, and adhesion to the substrate is also excellent. Pigments have also been investigated by scanning electron microscopy. The instrument is an excellent tool to determine particle size and shape of pigments, and therefore, can be used to determine the best pigment grades for making paints with specific properties.

Zinc oxide pigmented films prepared from linseed oils having low free fatty acid content swell less in water than equivalent films containing greater amounts of free fatty acid. An equation has been developed which appears to predict liquid surface tension in terms of nonpolar, polar, and hydrogen bonding forces. This equation should help in preparing improved pigments for use in linseed oil paints.

Copper, either in linseed oil (LSO) originally or introduced during handling, leads to dark-colored polyesteramides. The reddish-brown color component isolated by column chromatography from N,N-bis (2-hydroxyethyl) linseed amide (HELA) is a highly oxidized polymeric material containing copper. Light-colored polyesteramides were prepared from LSO by aminolysis with diethanolamine and direct esterification with dibasic acids; the isolation of HELA is thereby eliminated. These polymers dry rapidly to hard, clear, and colorless films. N-β-Methacryloxyethyl-N-ethyl linseed amide (EELAMA) and N,N-bis-(β-methacryloxyethyl) linseed amide (HELAMA) have been prepared. The reaction product of HELAMA with LSO at 230° C. has film properties superior to unreacted LSO. Free radical homo- and copolymerizations of HELAMA and EELAMA produced almost colorless, hard, glossy films. GLC coupled with mass spectrometry, NMR, and elemental analyses has given much information about the products formed in the reaction of hydrogen sulfide with unsaturated fatty esters under ultraviolet light at 25° C. Methyl oleate yields 9(10)mercapto stearate. Polyunsaturated esters yield mixtures that contain linear and cyclic mono and dithiols and cyclic sulfides.

Tris(triphenylphosphine)chlororhodium (catalyst) in methanol produces higher yields of conjugation in methyl linoleate than does the same amount of catalyst in chloroform. Deuterium studies show that methanol furnishes hydrogen to the catalyst which transfers it to the linoleate. Methanol is an active participant in the reaction and is therefore a superior medium to chloroform. The catalyst, however, has very limited solubility in alcohols.

Under a cooperative agreement at North Dakota State University, 1-alkenes were prepared by oxidative decarboxylation of the acid group in fatty acids with lead tetraacetate and cupric acetate. Stearic, oleic, and undecylenic acid readily converted to products that analyzed like 1-alkenes. Oxazolidones were prepared from phenyl isocyanate and hypobrominated oleic ester. Pyromellitic anhydride and dimer diisocyanate reacted in the presence of triethyl amine catalyst to give a foam-rubberlike product.

Publications

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Curing and Protecting Concrete

Cowan, J. C. 1969. Linseed oil coatings continue to show promise. Proc. 38th Annu. Flax Inst. of the U.S., Minneapolis, Minnesota, November 21-22, 1968, pp. 36-38.

2. Improved Durability of Linseed Oil Emulsion Paints

DeJarlais, W. J. 1969. A simple safety device for unattended laboratory heating. J. Chem. Educ. 46(5), p. 286.

Eissler, R. L., and Princen, L. H. 1970. Swelling of linseed oil films in acid and alkaline environments. J. Paint Technol. 42(542), pp. 155-158.

Gast, L. E., and Schneider, W. J. Dec. 23, 1969. Diisocyanate modified vegetable oil hydroxyl-terminated polyesteramide coatings. U.S. Patent 3,485,779.

Gast, L. E., Schneider, W. J., McManis, G. E., and Cowan, J. C. 1969. Polyesteramides from linseed and soybean oils for protective coatings: Diisocyanate-modified polymers. J. Amer. Oil Chem. Soc. 46(7), pp. 360-364.

McManis, G. E., Gast, L. E. 1969. Computer program to convert spectroreflectometric data into color designations. J. Paint Technol. 41(537), pp. 581-582.

Princen, L. H. 1969. Particle size analysis of coatings materials with the Coulter counter. Appl. Polym. Symp. (10), pp. 159-176.

Princen, L. H. Jan. 6, 1970. Linseed oil emulsion paints. U.S. Patent 3,488,202.

Rheineck, A. E., and Lakshmanan, P. R. (North Dakota State University, Fargo, North Dakota). 1969. Urethane coatings based on aldehyde oils. J. Amer. Oil Chem. Soc. 46(9), pp. 452-454.

Rheineck, A. E., and Lakshmanan, P. R. (North Dakota State University, Fargo, North Dakota). 1969. Reactions of aldehyde oils with a resinous polyol, styrene-allyl alcohol copolymer. J. Amer. Oil Chem. Soc. 46(9), pp. 455-458.

Rheineck, A. E., and Sreenivasan, B. (North Dakota State University, Fargo, North Dakota). 1969. Production of vinylic unsaturation by partial dehydrobromination of vicinal dibromo fatty derivatives. J. Amer. Oil Chem. Soc. 46(9), pp. 464-468.

CRAMBE UTILIZATION

Problems and Objectives

Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already exist for erucic acid as well as for imported rapeseed oil, which formerly was the only source of this acid. However, to insure optimum development of crambe as a new commercial crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and exploited. In addition, since economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals, suitable processes are required to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Developing new industrial chemical products from crambe oil.

2. Improving protein feed supplements from crambe.

Progress Report

A. NEW AND IMPROVED INDUSTRIAL AND FEED PRODUCTS

1. Industrial Nylons and Special-Purpose Plasticizers

Purpose: The high content (55-60%) of the 22-carbon monounsaturated acid, erucic acid, uniquely differentiates crambe oil from other domestic vegetable oils that contain 18-carbon acids almost exclusively. Chemical modification of erucic acid results in a variety of potentially useful products that cannot be conveniently obtained from any other starting material. Erucic acid derived from imported rapeseed oil is commercially available and enjoys a few well-established industrial uses. Domestically produced crambe oil should be a more economical source of erucic acid. However, to realize this potential, additional uses for crambe oil and erucic acid are needed. Present research at the Northern Division is concerned with nylons-13 and -1313, which are derived from brassylic acid obtained by ozonization of erucic acid, and with certain esters of brassylic and erucic acids as special purpose plasticizers.

Progress: Nylon-13 and -1313: Product yields are highest when methyl erucate is ozonized at low temperature and the ozonide is decomposed reductively. Under optimum conditions, nonanal is recovered in 80-90 percent yield, but methyl 12-formyldodecanoate yields are 10-30 percent lower. Over-oxidation and polymerization of intermediates are presumed responsible for the reduced yields of aldehyde-ester. Undesirable byproducts are a serious problem at higher temperatures in dichloromethane or methanol, but reactions in mixtures of propionic acid and an alcohol are relatively unaffected by temperature changes between 0° and 24° C. These mixed systems show promise for use in preparing nylon-13 monomer. New methods of a separation have been developed that should be useful in preparing for further study experimental quantities of the byproduct polymers formed in small amounts during oxidative ozonolysis of erucic acid. Methods have been developed for measuring the color of nylon-1313, brassylic acid, and 1,13-diaminotridecane. Preliminary minimum specifications on the color of nylon-1313 and on the color and color stability of brassylic acid and the diamine have been determined.

Special-Purpose Plasticizers: One hundred additional films were cast from mixtures of selected homopolymers and from copolymers of alkyl vinyl brassylates and vinyl chloride. A total of 152 externally and internally plasticized films are to be evaluated. A second commercial poly(vinyl chloride) (PVC) resin was also used to validate any differences observed between external and internal plasticizations of PVC. Bis(2-methylpentyl) brassylate, the most efficient brassylate diester, was added to PVC as an external plasticizer reference for the copolymers of 2-methylpentyl vinyl brassylate and vinyl chloride. The commercially successful bis(2-ethylhexyl) sebacate was also used for preparing reference films. A strip from each film was examined spectrophotometrically over the entire range of UV, visible, near IR, and IR. Unlike bis(2-ethylhexyl) phthalate-plasticized PVC, copolymers of vinyl chloride and alkyl vinyl brassylate were transparent in the UV region, 220-350 nm. PVC itself absorbed strongly below 210 nm. Plasticizer composition could be verified in the IR region where characteristic peaks (e.g. carbonyl) absorbed in proportion to plasticizer content.

Polymerization studies on distilled allyl esters of erucic acid and of mono- and dicarboxylic acids derived from it or from crambe oil showed all monomers to react readily under bulk polymerization conditions. For monocarboxylic acid esters, the highest degrees of polymerization and conversion to polymer were achieved at 120° C. with tertiary-butyl perbenzoate as initiator. Allyl brassylate and allyl azelao-brassylate were very efficient in PVC external plasticization, but their volatility and light instability detracted from their performance. All monocarboxylic acid allyl esters were incompatible with PVC.

Under a PL 480 grant to the Institute of General Chemistry, Warsaw, Poland, 1-monoerucin and 1-monoolein were prepared by reaction of erucic or oleic acid with 1,2-isopropylideneglycerol and subsequent removal of the blocking group. The monoglycerides were converted to 1-eruco-2,3-diolein and 1-oleo-2,3-dierucin by reaction with oleoyl chloride and erucoyl chloride, respectively.

2. Protein Feed Supplements

Purpose: After extraction of the oil from crambe seed, the residue contains a large percentage of high-quality protein. However, like certain other oilseed meals, crambe meal contains antinutritional factors that restrict its utilization as an animal feed supplement and, therefore, limit its price value. As a result, expansion to maximum market potential is curtailed since the oil must sell for a higher price to compensate for restricted usage and lower value of the meal. Methods of processing crambe are sought that will remove or deactivate antinutritional factors in crambe meal and thereby provide a protein supplement having maximum nutritional quality and acceptability.

Progress: Amino acid composition and results of a rat feeding study indicate that defatted meal produced after water extraction of dehulled intact crambe seed is of exceptionally high nutritional quality. Factors affecting cost and efficiency of a process based on the water extraction procedure have been investigated. Stepwise extraction of one part of the seed with a total of 7 to 11 volumes of hot water removes 89 to 96 percent of the epi-progoitrin (which comprises 90% of the total glucosinolates in crambe seed). Chick feeding of the pelleted and reground meal from the extracted seeds as 17.5 and 35 percent of the ration gave respective weight gains of 95 and 88 percent of the weight of the controls.

No completely satisfactory nonextractive treatment has been found for detoxifying the meal. However, in engineering experiments, moist cooking of crambe meal with acidic or basic additives improves crambe meal quality very markedly. These represent economical approaches. Complete removal of the toxic factors from defatted flakes by water extraction gives a meal of highest biological quality but is a more costly approach. Water extraction also generates a waste disposal problem. Further studies are needed to determine the application and feasibility of the various methods of treating crambe meal.

Crude crambe thioglucosidase responded as a sulfhydryl enzyme to p-chloromercuribenzenesulfonate and is reactivated by 2-mercaptoethanol or ascorbate. Sequential fractionation by ammonium sulfate precipitation and gel filtration chromatography led to an enzyme preparation with a specific activity fivefold higher than previously reported. Enzymatic production of 1-cyano-2(S)-hydroxy-3,4-epithiobutanes

has been achieved for the first time apart from crambe meal. Soluble thioglucosidase prepared without the addition of reducing agents is labile, but will produce the epithiobutane as well as 1-cyano-2(S)-hydroxy-3-butene from epi-progoitrin in the presence of ferrous ion.

Publications

A. NEW AND IMPROVED INDUSTRIAL AND FEED PRODUCTS

1. Industrial Nylons and Special-Purpose Plasticizers

Bagby, M. O., Smith, C. R., Jr., and Wolff, I. A. 1969. Olefin-tetracyanoethylene oxide adducts and some of their derivatives. J. Org. Chem. 34(9), pp. 2732-2735.

Greene, J. L., Jr.,[1] Burks, R. E., Jr.,[1] and Wolff, I. A. ([1]Southern Research Institute, Birmingham, Alabama). 1969. 13-Aminotridecanoic acid from erucic acid. Ind. Eng. Chem., Prod. Res. Develop. 8(2), pp. 171-176.

Mod, R. R.,[1] Magne, F. C.,[1] Skau, E. L.,[1] Nieschlag, H. J., Tallent, W. H., and Wolff, I. A. ([1]South. Util. Res. Develop. Div., New Orleans, Louisiana). 1969. Preparation and plasticizing characteristics of some N,N-disubstituted amides of erucic and crambe acids. Ind. Eng. Chem., Prod. Res. Develop. 8(2), pp. 176-182.

Nieschlag, H. J., Tallent, W. H., and Wolff, I. A. 1969. Simplified preparation of a diester plasticizer from crambe seed oil. Ind. Eng. Chem., Prod. Res. Develop. 8(2), p. 216.

Perkins, R. B.,[1] Roden, J. J. III,[1] Tanquary, A. C.,[1] and Wolff, I. A. ([1]Southern Research Institute, Birmingham, Alabama). 1969. Nylons from vegetable oils: -13, -13/13, and -6/13. Mod. Plast. 46(5), pp. 136-137, 140, 142.

2. Protein Feed Supplements

Carlson, K. D., Smith, C. R., Jr., and Wolff, I. A. 1970. Unsaturated nitro sugars. Preparation of 4-O-acetyl-1,2-dideoxy-3,5-O-ethylidene-1-nitro-D-erythro-pent-1-enitol. Carbohyd. Res. 13(3), pp. 391-402.

Carlson, K. D., Smith, C. R., Jr., and Wolff, I. A. 1970. N.M.R. spectra and benzene-induced solvent shifts. Acetylated carbohydrates substituted with 1,3-O-ethylidene or nitro groups. Carbohyd. Res. 13(3), pp. 403-415.

VanEtten, C. H. 1969. Goitrogens. In "Toxic Constituents of Plant Foodstuffs," ed. I. E. Liener, chap. 4, pp. 103-142. New York.

VanEtten, C. H., Daxenbichler, M. E., and Wolff, I. A. 1969. Natural glucosinolates (thioglucosides) in foods and feeds. J. Agr. Food Chem. 17(3), pp. 483-491.

NEW CROPS UTILIZATION

Problems and Objectives

Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. To develop a new crop, three basic steps are involved: (1) survey of wild plants to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential. Close cooperation is needed with plant scientists who provide assistance and advice in acquisition and selection of samples for screening and who have responsibility for subsequent agronomic research such as establishing proper cultural practices and identifying the best strains and varieties.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Identify new plant sources for industrial vegetable oils from among samples collected worldwide.

2. Determine utilization potential of oils and feed meals from selected new oilseeds.

3. Produce marketable rotenoid products from the domestic legume Tephrosia vogelii.

4. Produce papermaking pulp from kenaf.

Progress Report

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. New Sources of Industrial Vegetable Oils

Purpose: In the last 12 years at the Northern Division, some 8,800 seed samples from about 5,400 plant species have been chemically investigated, with emphasis on the nature of the seed oils. As a result of this research, numerous species are under agronomic evaluation as potential new crops; one new oilseed (crambe) has entered commercial production, and basic findings of high significance in natural products chemistry have been made. Fifty fatty acids not known to occur in

seed oils, or not known to occur at all in nature, have been discovered, isolated, and fully identified. Species have been found that produce seed oils like those of (imported) coconut, palm kernel, castor, tung, and rapeseed. Also, additional species were found that had seed oils like those of flaxseed and safflower, some of which may prove more widely adapted or more productive than those present crops. Many of the seeds and oil-free meals were found rich in protein; a number contained water-soluble gums of value as paper additives. Examination of 5,400 species represents a significant effort but is only a beginning toward the opportunity of finding valuable chemicals among the 250,000 known species of higher plants. The continuing high rate of discovery of lipids having new and unusual structures among previously uninvestigated species offers promise of equally rewarding results from future investigations.

Progress: A new hydroxy acid, 14-hydroxy-cis-11,cis-17-eicosadienoic acid, was discovered in oil from Lesquerella auriculata. Oil from Stokesia laevis was found to contain 70 percent vernolic acid. New sources were found for eleostearic acid, acetotriglycerides, parinaric acid, and hexadecenoic acid. Altogether 525 seed samples were examined for oil and protein content, making a total of 3,037 samples so analyzed in the program thus far. A rapid method was developed for determining gum in guar seed. Among 341 species examined for L-dopa, only Mucuna (syn. Stizolobium) seed contained significant amounts (more than 1%).

Cyanolipids, a new class of compounds, have been shown to occur extensively in seed oils of the Sapindaceae and frequently are major constituents of these oils. 13-Hydroxy-trans-9-octadecenoic acid has been isolated from Monnina emarginata seed oil, another new acid in addition to three others isolated previously from this source /(S)-coriolic, 13-oxo-trans-9,trans-11-octadecadienoic, and 13-oxo-trans-9-octadecenoic acids/. A C_{17}-homolog of sterculic and malvalic acids has been isolated from Pavonia sepium seed oil.

Under a cooperative agreement with Oregon State University, Corvallis, Oregon, plantings of Brassica were made in October for the purpose of developing inbred lines, crossing desirable combinations, determining production potential, testing adaptation, and evaluating progeny from previously made crosses.

2. Rotenoids From Tephrosia vogelii

Purpose: As an insecticidal and piscicidal agent, rotenone has the widely recognized advantages of lack of persistence, failure to induce biological resistance, and reputation for low toxicity to mammals. For these reasons, rotenone-containing formulations enjoy a degree of consumer preference for home use, in gardens, and for clearing lakes

also been found active and have been shown to be structually related esters. Approximately 1,000 pounds of Cephalotaxus harringtonia trees were received from a nursery in Portland, Oregon. Processing was initiated to provide sufficient harringtonine to get preclinical pharmacology studies underway at the National Institutes of Health.

Publications

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. New Sources of Industrial Vegetable Oils

Berry, C. D.,[1] Lessman, K. J.,[1] White, G. A.,[2] and Earle, F. R. ([1]Purdue University, Lafayette, Indiana; [2]USDA Crops Research Div., Beltsville, Maryland). 1970. Genetic diversity inherent in Vernonia anthelmintica (L.) Willd. Crop Sci. 10(2), pp. 178-180.

Kleiman, R., Earle, F. R., Tallent, W. H., and Wolff, I. A. 1970. Retarded hydrolysis by pancreatic lipase of seed oils with trans-3-unsaturation. Lipids 5(6), pp. 513-518.

Kleiman, R., Earle, F. R., and Wolff, I. A. 1969. Wax esters from sunflower oil tank settlings. J. Amer. Oil Chem. Soc. 46(9), p. 505.

Kleiman, R., Earle, F. R., and Wolff, I. A. 1969. Dihydrosterculic acid, a major fatty acid component of Euphoria longana seed oil. Lipids 4(5), pp. 317-320.

Mikolajczak, K. L., Seigler, D. S., Smith, C. R., Jr., Wolff, I. A., and Bates, R. B.[1] ([1]University of Arizona, Tucson, Arizona). 1969. A cyanogenetic lipid from Cordia verbenacea DC. seed oil. Lipids 4(6), pp. 617-619.

Mikolajczak, K. L., Smith, C. R., Jr., and Wolff, I. A. 1970. Phenolic and sugar components of Armavirec variety sunflower (Helianthus annuus) seed meal. J. Agr. Food Chem. 18(1), pp. 27-32.

Mikolajczak, K. L., Smith, C. R., Jr., and Wolff, I. A. 1970. Stability of crude sunflower oils to autoxidation and to seed aging. J. Amer. Oil Chem. Soc. 47(1), pp. 24-25.

Phillips, B. E., Smith, C. R., Jr., and Hagemann, J. W. 1969. Glyceride structure of Erlangea tomentosa seed oil, a new source of vernolic acid. Lipids 4(6), pp. 473-477.

Phillips, B. E., Smith, C. R., Jr., and Tjarks, L. W. 1970. (S)-13-Hydroxy-cis-9,trans-11-octadecadienoic acid lactone, a 14-membered ring compound from Monnina emarginata seed oil. J. Org. Chem. 35(6), pp. 1916-1919.

and ponds of rough fish before restocking. The quantity of rotenoids sold each year in the U.S. varies markedly because the cost of rotenoids, which are obtained from foreign sources, is high and unstable in comparison to domestic synthetic competitive products and because of fluctuations and uncertainties in supply. A satisfactory domestic source of rotenoids would eliminate these disadvantages and promote the use of rotenoids in preference to synthetics that present health and pollution hazards.

Tephrosia vogelii is a plant which will grow in the United States and contains rotenoids in the above-ground parts, which are amenable to machine harvesting. This legume has been recognized for several years as a potential domestic source of rotenoids. Realization of this potential has been hindered by lack of a suitable analytical method to follow necessary plant selection and agronomic and processing studies. Also, an economical process for fractionation of T. vogelii is needed to ensure optimum recovery of a product with maximum rotenoid content. Research at the Northern Division is directed towards solution of these analytical and processing problems.

Progress: Rotenoids can be extracted efficiently from dry powders of Tephrosia vogelii leaves. Chloroform, methylene chloride, and acetone were the most effective of 11 solvents for extraction by steeping at room temperature. Hexane was least effective; yet aqueous hexane gave the purest extracts. With fresh leaflets, which yield up to 20 percent more total rotenoids than dried material, hexane is as effective as acetone in Soxhlet extractions. Freezing leaflets reduces rotenone yield but does not significantly alter the amount of extractable deguelin. Rotenone and deguelin can be separated from tephrosin and several other contaminating materials present in crude extracts by preparative-scale chromatography on silica gel. Deguelin of 70 to 90 percent purity (ultraviolet analysis) has been isolated from a rotenone-deficient variety of T. vogelii. Plants of 23 varieties representing 17 species have been grown, collected, and separated into their component parts for study of their content of individual rotenoids.

3. Kenaf for Paper

Purpose: Kenaf is an annual pulp crop that shows great promise as a supplement to, or replacement for, Southern hardwood pulpwood. However, despite research conducted to date, information gaps exist in many aspects of its production and utilization. Consequently, it is not yet possible to specify a completely satisfactory and practical sequence of operations for proceeding from kenaf in the field through its mill conversion to fully acceptable commercial-grade papers. Some of the areas requiring further study include removal of undesirable constituents, continuous pulping, technology for pulping green, field-dried or preserved kenaf, bleaching, and refining.

Powell, R. G., Kleiman, R., and Smith, C. R., Jr. 1969. New sources of 9-D-hydroxy-cis-12-octadecenoic acid. Lipids 4(6), pp. 450-453.

Seigler, D. S., Mikolajczak, K. L., Smith, C. R., Jr., and Wolff, I. A. 1970. Structure and reactions of a cyanogenetic lipid from Cordia verbenacea DC. seed oil. Chem. Phys. Lipids 4(2), pp. 147-161.

Smith, C. R., Jr. 1970. Optically active long-chain compounds and their absolute configurations. In "Topics in Lipid Chemistry," ed. F. D. Gunstone, vol. 1, pp. 277-368. London.

Smith, C. R., Jr., Freidinger, R. M., Hagemann, J. W., Spencer, G. F., and Wolff, I. A. 1969. Teucrium depressum seed oil: A new source of fatty acids with Δ^5-unsaturation. Lipids 4(6), pp. 462-465.

Spencer, G. F., Kleiman, R., Earle, F. R., and Wolff, I. A. 1969. Rapid micromethod for location of ene-yne and α-hydroxy conjugated diene systems in straight-chain compounds. Anal. Chem. 41(13), pp. 1874-1876.

Spencer, G. F., Kleiman, R., Earle, F. R., and Wolff, I. A. 1970. Unusual olefinic fatty acids in seed oils from two genera in the Ranunculaceae. Lipids 5(2), pp. 277-278.

Spencer, G. F., Kleiman, R., Earle, F. R., and Wolff, I. A. 1970. The trans-6 fatty acids of Picramnia sellowii seed oil. Lipids 5(3), pp. 285-287.

Tookey, H. L., and Gentry, H. S.[1] ([1]USDA Crops Research Div., Beltsville, Maryland). 1969. Proteinase of Jarilla chocola, a relative of papaya. Phytochemistry 8(6), pp. 989-991.

2. Rotenoids From Tephrosia vogelii

Carlson, D. G., and Tallent, W. H. 1970. Gas-liquid chromatographic determination of rotenone and deguelin in extracts of Tephrosia vogelii. J. Chromatogr. Sci. 8(5), pp. 276-278.

Delfel, N. E., Tallent, W. H., Carlson, D. G., and Wolff, I. A. 1970. Distribution of rotenone and deguelin in Tephrosia vogelii and separation of rotenoid-rich fractions. J. Agr. Food Chem. 18(3), pp. 385-390.

3. Kenaf for Paper

Clark, T. F. 1970. First bond paper made using kenaf pulp in the furnish. Paper Trade J. 154(2), pp. 48-49.

Progress: Successful production of two lots of commercial-type bond paper was achieved on the Northern Division's Fourdrinier papermaking machine. Furnishes contained 40 percent of pulps prepared experimentally from green and field-dried kenaf by pilot-plant-scale continuous sulfate cooking. Evaluation of some sulfate pulps prepared from baled kenaf after 1-1/2 years' storage indicated that yields (based on material charged to digester) of screened pulps ranging from 44 to 48 percent (compared to 48 to 50 percent for fresh kenaf) with retention of most favorable qualities could be realized.

A research contract was negotiated late in FY 1970 with the Herty Foundation, Savannah, Georgia, providing for studies on pilot-plant production of chemical pulp from kenaf. This research is expected to provide much technical and economic information needed for accurate appraisal by industry of the commercial potential of kenaf.

4. Tumor-Inhibiting Substances

Purpose: Present methods of treatment of cancer include surgery, exposure to radiation, and chemotherapy. Of these treatments, the chemotherapeutic approach is the least developed; yet it affords the greatest ultimate hope for some cases of internal cancer, including leukemia, which are essentially hopeless at present.

Thousands of samples of plant seeds representing an unusually diverse botanical spectrum are available at the Northern Division. These seeds were acquired for a chemical screening program directed towards new industrial crops. The very limited testing that has so far been possible with extracts of these seeds has revealed "confirmed actives" in tests conducted under the direction of the Cancer Chemotherapy National Service Center. One of the "confirmed actives" was an extract of the seed of a yew-like evergreen shrub, Cephalotaxus harringtonia. The active principle, which also occurs in other parts of the plant, is an alkaloid that has been isolated and named "harringtonine." This new compound slows the progress of experimental leukemia in laboratory mice. It is effective at very low dosages, well below the level of toxicity to the animals. Under preferred conditions of administering the drug, treated leukemic mice lived more than twice as long as untreated mice. Harringtonine is undergoing more detailed chemical and pharmacological study.

Progress: Harringtonine has been characterized as an ester of 3-carbomethoxy-3,6-dihydroxy-6-methylheptanoic acid. The identification of harringtonine and several of its derivatives was aided by high resolution mass spectrometry. The molecular weight of harringtonine was determined to within 1 millimass unit thus making it possible to calculate the atomic formula directly. Three congeners of harringtonine have

Clark, T. F., and Wolff, I. A. 1969. A search for new fiber crops. XI. Compositional characteristics of Illinois kenaf at several population densities and maturities. Tappi 52(11), pp. 2111-2116.

White, G. A.,[1] Cummins, D. G.,[2] Whiteley, E. L.,[3] Fike, W. T.,[4] Greig, J. K.,[5] Martin, J. A.,[6] Killinger, G. B.,[7] Higgins, J. J.,[8], and Clark, T. F. ([1]USDA Crops Research Div., Beltsville, Maryland; [2]University of Georgia, Experiment, Georgia; [3]Texas A&M University, College Station, Texas; [4]North Carolina State University, Raleigh, North Carolina; [5]Kansas State University, Manhattan, Kansas; [6]Clemson University, Clemson, South Carolina; [7]University of Florida, Gainsville, Florida; [8]USDA Crops Research Div., Glenn Dale, Maryland). 1970. Cultural and harvesting methods for kenaf...An annual crop source of pulp in the southeast. USDA Agr. Res. Serv. Prod. Res. Rep. No. 113, 38 pp.

4. Tumor-Inhibiting Substances

Perdue, R. E., Jr.,[1] Spetzman, L. A.,[1] and Powell, R. G. ([1]USDA Crops Research Div., Beltsville, Maryland). 1970. Cephalotaxus--Source of harringtonine, a promising new anti-cancer alkaloid. Amer. Hort. Mag. 49(1), pp. 19-22.

Powell, R. G., Weisleder, D., Smith, C. R., Jr., and Rohwedder, W. K. 1970. Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahedron Lett. (11), pp. 815-818.

Powell, R. G., Weisleder, D., Smith, C. R., Jr., and Wolff, I. A. 1969. Structure of cephalotaxine and related alkaloids. Tetrahedron Lett. (46), pp. 4081-4084.

FORAGE UTILIZATION (NORTHERN REGION)

Problems and Objectives

Tall fescue grass is grown extensively in the Southeast, in the Intermountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. Thirty-five to fifty million acres of fescue are grown for forage use in the southeastern part of the United States alone. Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks, animals become emaciated and frequently die. Elimination of this disease would prevent an estimated average economic loss of about $5 million annually.

The major objectives of current research are to discover the cause of fescue foot disease and to develop and evaluate alternate ways for its prevention.

Progress Report

A. NEW AND IMPROVED FEED PRODUCTS

1. Mycotoxins Associated With Tall Fescue

Purpose: Initial studies on the cause of toxicity of tall fescue emphasized the preparation and fractionation of various extracts of tall fescue plants from toxic fields. However, the sporadic nature of fescue foot outbreaks then suggested involvement of fungi in the etiology of the disease. Extracts of cultures of several fungi isolated from samples of fescue from toxic fields were found to produce toxic responses in rabbits and mice. In current research, fungi found on toxic fescue are being studied to determine those producing mycotoxins. These mycotoxins are concentrated or isolated and their effects on test animals are determined.

Progress: All but one of the 28 *Fusarium* isolates toxic or questionably toxic to mice produce either 4-acetamido-4-hydroxy-2-butenoic acid γ-lactone (butenolide) or 4β,15-diacetoxy-8α-(3-methylbutyryloxy)-12, 13-epoxytrichothec-9-en-3α-ol (T-2), or both.

A total of 1,400 g. of the *F. tricinctum* mycotoxin butenolide was prepared synthetically. Oral daily dosage to heifers at 3 or 2 g. per 100 pounds proved lethal in 2 or 3 days, respectively. In tests conducted by cooperating scientists at the Wisconsin Agricultural Experiment Station, oral daily dosage to two heifers at 1 g. per 100 pounds

for 60-80 days produced necrosis of the distal 1-1/2-inch (approximately) portion of the tail and a 15 percent loss in body weight. These animals were on a ration of fescue hay believed to be nontoxic, had their tail switches clipped, and were left outdoors at Madison, Wisconsin, during the recent extreme winter. The control animal, also on the same ration, maintained its body weight but developed a necrotic tail tip (7/8"), possibly due to frost bite. Up to a 5-gram equivalent of T-2 toxin can be recovered per kilogram of substrate fermented with Fusarium tricinctum. Over 6.5 grams of pure crystalline T-2 toxin has been produced for a dose-response experiment in cattle.

Publications

A. NEW AND IMPROVED FEED PRODUCTS

1. Mycotoxins Associated With Tall Fescue

Gratz, J. A., Cook, C. E., and Wall, M. E. (Research Triangle Institute, Research Triangle Park, North Carolina). 1970. DL-4-Acetamido-4-hydroxy-2-butenoic acid γ-lactone. Org. Prep. Proced. 2(1), pp. 51-52.

Yates, S. G., Tookey, H. L., and Ellis, J. J. 1970. Survey of tall-fescue pasture: Correlation of toxicity of Fusarium isolates to known toxins. Appl. Microbiol. 19(1), pp. 103-105.

Yates, S. G., Tookey, H. L., Ellis, J. J., Tallent, W. H., and Wolff, I. A. 1969. Mycotoxins as a possible cause of fescue toxicity. J. Agr. Food Chem. 17(3), pp. 437-442.